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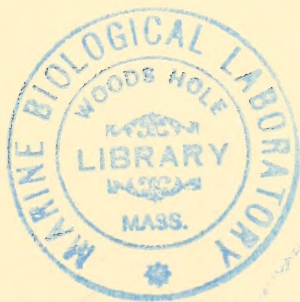
ADVENTURES IN
RADIOISOTOPE RESEARCH

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ADVENTURES IN RADIOISOTOPE RESEARCH

The Collected Papers of
GEORGE HEVESY
in Two Volumes

VOLUME TWO



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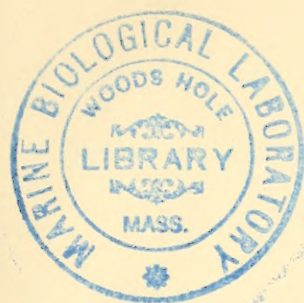


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51. A METHOD OF BLOOD VOLUME DETERMINATION

L. HAHN AND G. HEVESY

From the Institute of Theoretical Physics, Copenhagen

THE method usually applied in the determination of blood volume is that worked out by ROWNTREE and his colleagues (1929). The principle of the method is that a dyestuff is injected intravenously and its degree of dilution determined⁽¹⁾. As the dye only mixes with plasma, the volume of the plasma alone is thus measured. The relative volume of corpuscles and plasma is determined with the haematocrit. To arrive at the blood volume, the volume of the corpuscles is added to that of the plasma.

ROWNTREE gives the following description of the method applied (comp. also FLEISCHER-HANSEN, 1928). A 1.5 per cent solution of vital red in distilled water is prepared. Four centrifuge tubes are provided and 1 cc. of a 1.6 per cent solution of sodium oxalate is placed into each of them. A needle is inserted in the vein of one arm and 10 cc. of blood are removed. 5 cc. are placed into each of two centrifuge tubes for standard plasma colour. The dye is then injected. After 3 to 6 min, 10 cc. of blood are withdrawn from the vein of the other arm and 5 cc. placed into each of the two remaining centrifuge tubes. All four tubes are centrifuged and the relative volume of corpuscles and plasma measured. The second sample is compared with a known strength of the dye and the degree of dilution of the dye in the plasma is thus obtained.

When considering the possible errors of this method, the main question at issue is whether, when the second sample is collected, the dye is uniformly mixed in the plasma and none has yet escaped into the tissue spaces or urine, a further possible source of error being the adsorption of a part of the dye by the enormous surface of the capillary wall.

⁽¹⁾ Instead of a dyestuff, diphteria antitoxin was used in some determinations (v. BEHRING, 1912; MADSEN, 1934).

DETERMINATION OF BLOOD VOLUME BASED ON THE DILUTION OF LABELLED CORPUSCLES

In this note, we wish to describe a method of blood volume determination based on an entirely different principle from that described above. We inject into the vein of a rabbit A a known volume of labelled corpuscles taken from another rabbit B and determine the extent to which these labelled corpuscles are diluted in the circulation of rabbit A. Labelled corpuscles of rabbit B are obtained in the following way. We administer by subcutaneous injection some labelled (radioactive) sodium phosphate to rabbit B. In the course of about a week, a substantial fraction of the phosphatide molecules of the bone marrow and other organs are renewed. As this renewal takes place in the presence of labelled phosphate, the newly formed phosphatide molecules will contain labelled P atoms. Corpuscles formed in a medium containing labelled phosphatide molecules will necessarily incorporate some of them. Labelled phosphatide molecules can also enter to some extent into the corpuscles by exchange of non-active phosphatide molecules with active phosphatide molecules present in the plasma. The various ways of incorporating labelled phosphatide into corpuscles are described in detail in a paper which is in print (HAHN and HEVESY, 1940).

Besides labelled phosphatides, labelled varieties of several acid-soluble organic phosphorus compounds as, for example, those of glycerophosphate and adenosintriphosphate, are found in the corpuscles. Each of these compounds can be used as an indicator when determining the dilution of the corpuscles of rabbit B in the circulation of rabbit A. It is, however, more convenient to extract the total acid-soluble P and to use the mixture obtained as an indicator.

DETERMINATION OF THE BLOOD VOLUME OF A RABBIT WEIGHING 2 kgm

a) Making use of the labelled phosphatides of the corpuscles

We administered radioactive sodium phosphate of negligible weight having the activity of about 0.001 milliCurie to rabbit B. After the lapse of a week, 1 cc. of blood of rabbit B containing 0.32 cc. corpuscles was injected into the jugularis of rabbit A. After the lapse of 5 min, 50 cc. blood were collected and, after the addition of heparin, centrifuged. The haematocrit value of this sample was found to be 0.33. The phosphatides of the corpuscles were thoroughly extracted by BLOOR's method. Their P was converted by wet ashing into phosphate. The phosphate was precipitated as ammonium magnesium salt. Before precipitation, sodium phosphate was added to the solution to obtain a precipitate of about 80 mgm. The activity of the precipitate was then determined by means of a GEIGER counter. The comparison of the acti-

vity of the samples is facilitated if they have practically the same weight and it is for this reason that we added to the original sample a comparatively large amount of non-active phosphate. The corpuscles of 1 cc. of the blood of rabbit B were also extracted with ether-alcohol and the extract treated in the way described above. The activity of the sample thus obtained, was then compared with that of the corresponding sample of rabbit A.

Let us denote the injected blood volume by V_I , the volume of the sample collected for analysis from rabbit B and rabbit A, respectively, by V_I and V_{II} , and the activity of the two samples obtained by A_I and A_{II} ; then the blood volume to be determined (X) becomes

$$X = \frac{A_I \cdot V_{II} \cdot V_I}{A_{II} \cdot V_I} - V_I.$$

In some of our experiments, before injecting, for example, 1 cc. blood into the jugularis of rabbit A, we removed 1 cc. In that case, the second term of the equation becomes 0.

Operations involved in the determination of the total blood volume are thus: measurement of the volume of three samples, and the comparison of the radioactivity of two samples.

In the above mentioned experiment the corpuscle phosphatides of 1 cc. blood of rabbit B contained 100 relative activity units; the activity of the corpuscle phosphatides extracted from 50 cc. blood of rabbit A was found to be 53.3. From these values it follows that the blood volume of rabbit B amounts to $93.8 - 1 \text{ cc.} = 92.8$.

b) Making use of labelled acid-soluble compounds of the corpuscles

We can check the result obtained above by another procedure in which, instead of the labelled phosphatides, the labelled acid-soluble phosphorus compounds are involved. After extraction of the phosphatides, the corpuscles are extracted with 10 per cent trichloroacetic acid. The P of the filtrate obtained is converted, as described above, into ammonium magnesium phosphate. The activity of the sample obtained from rabbit A is compared with that of the sample from rabbit B, as described above. The figures obtained being 100 and 54.4 respectively, the total blood volume of rabbit A becomes

$$X = \frac{A_I \cdot V_{II} \cdot V_I}{A_{II} \cdot V_I} = V_I = \frac{100 \cdot 50 \cdot 1}{54.4 \cdot 1} - 1 = 91.9 - 1 = 90.9$$

The use of labelled acid-soluble P compounds leads thus to practically the same result as obtained with labelled phosphatides as indicators.

The blood volume per kgm of rabbit weight was found, in the experiment described above, to be 46 cc. In another experiment the value of 38 cc. was found.

DETERMINATION OF THE BLOOD VOLUME OF A CHICK WEIGHING 135 gm

Labelled sodium phosphate was administered to chick B and, 24 hours later, 0.5 cc. blood of this chick was injected into the jugular vein of chick A. The haematocrit values were found to be 0.26 and 0.28, respectively. We found the activity of the phosphatides extracted from the corpuscles of 1 cc. blood of chick A to be 7.6, taking that of the phosphatides secured from the corpuscles of 1 cc. blood of chick B to be 100. The total blood volume of chick A is thus

$$X = \frac{100 \cdot 1 \cdot 0.5}{7.6 \cdot 1} - 0.5 = 6.1,$$

or 45 cc. per kgm weight.

LOSS OF LABELLED P COMPOUNDS BY THE CORPUSCLES

We wish now to discuss the possible loss of labelled P compounds by the corpuscles during the interval between the injection of labelled corpuscles of rabbit B into the circulation of rabbit A and the securing of blood samples of rabbit A. Such a loss would clearly involve a source of error by leading to too high values for the blood volume to be determined. As to the loss of labelled phosphatides, we found that the labelled phosphatide content of the corpuscles of rabbit A, after the lapse of 1 hour, amounts to 90 per cent of that found after the lapse of 6 min. This result shows that the disappearance of labelled phosphatides from the corpuscles takes place at a slow rate.

As to the disappearance of the labelled acid-soluble P molecules from the corpuscles, we find the following result. The labelled acid-soluble P content of 1 cc. corpuscle of rabbit A, 8.5 min after injecting the blood of rabbit B into rabbit A, is, as seen in Table 1 and Fig. 1, within the errors of experiment, identical with that found after 2.5 min.

We have now to investigate if, during the lapse of 5 min or more, after which time blood samples in the experiments described in this paper were secured, a uniform mixing of the labelled corpuscles of rabbit B in the circulation of rabbit A took place. From the data contained in Table 1 and Fig. 1 we can conclude that, already after the lapse of 2.5 min., a uniform mixing actually took place. Such a result in the case of a small animal with fast circulation could be expected since, in experiments carried out on human subjects, a uniform mixing of dyestuffs injected into the plasma was found to take place in the course of 6 min. (ROWNTREE *et alia*, 1929; GRAFF *et alia*, 1931).

TABLE 1. — DISAPPEARANCE OF LABELLED ACID-SOLUBLE P COMPOUNDS FROM THE CORPUSCLES

Time elapsed after injecting the blood of rabbit B into rabbit A	Per cent of labelled acid-soluble P injected, present in 1 cc. corpuscle of rabbit A
2.5 min	3.39
5 "	3.21
6.5 "	3.39
8.5 "	3.30
26.5 "	3.12
60 "	2.88
120 "	2.58
210 "	1.35

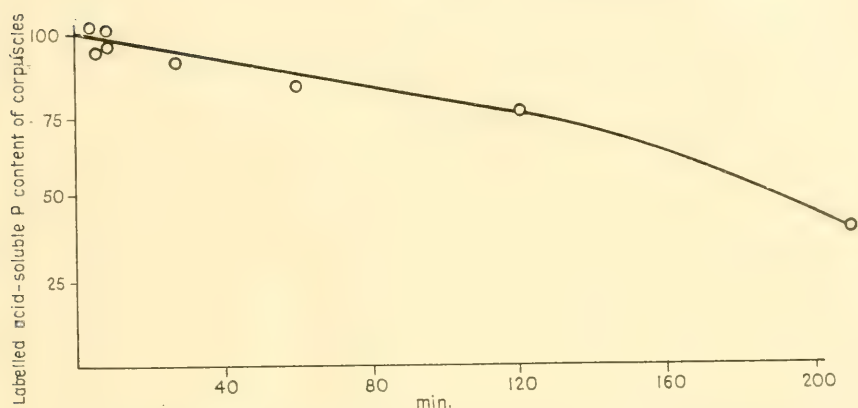


FIG. 1. Disappearance of labelled acid-soluble P from corpuscles, injected into the circulation of a rabbit.

DISCUSSION

The sole difference between the normal and the labelled corpuscles is that in some of the molecules present in the normal ones P (having the mass number 31) is replaced by radioactive P (having the mass number 32). In 1 cc. corpuscles of rabbit B, 0.06 mgm phosphatide P was present. Of these 0.06 mgm, 10^{-11} mgm were radioactive ^{32}P atoms. As 1 cc. contains about $3 \cdot 10^9$ corpuscles, one corpuscle contains on an average 10^{-21} mgm ^{32}P or only about one corpuscle in a hundred contained an active phosphatide molecule. After injecting the blood sample of rabbit B into rabbit A, a strong dilution of the labelled phosphatides took place: only one in about three thousand corpuscles now contains a radioactive phosphatide P atom. The replacement of a minute percentage of the ^{31}P atoms by ^{32}P atoms in the P compounds of the corpuscles can hardly influence to any noticeable extent the chemical properties of the corpuscles and

we can, therefore, claim that, when applying the method described in this note, no non-physiological component is introduced into the circulation. As to the β -radiation emitted by the ^{32}P atoms present in the corpuscle phosphatides, the number of β -particles emitted per minute in the total circulation of rabbit A amounts to only about 1000, while the number emitted by the total acid-soluble fraction amounts to about 30 times that figure. How insignificant these figures are, can best be realized when we envisage that this radiation corresponds to that of only 10^{-8} and 10^{-9} gm radium, respectively.

When carrying out experiments as those described above on human subjects, it may be advisable to make use of the acid-soluble P compounds of the corpuscles as indicators. Since, in this case, one may use less radioactive P, such experiments can be carried out on human subjects by administering by subcutaneous injection or by mouth to the blood donor sodium phosphate having a β -radioactivity corresponding to that of about 0.01 milliCurie or even less.

Summary

A method of blood volume determination based on the determination of the dilution of labelled corpuscles is described. Radioactive sodium phosphate is administered to rabbit B; after the lapse of some days, a known blood sample of this rabbit is injected into the vein of rabbit A. A few minutes later, the corpuscles of a known volume of the blood of rabbit A are secured, their phosphatide content extracted, and its activity measured. Corpuscles of a known blood volume of rabbit B are treated in the same way. From the ratio of the labelled phosphatide P content of the corpuscles of rabbit A and rabbit B, the total blood volume of rabbit A is calculated.

An alternative and often preferable determination is based on the comparison of the activity of the acid-soluble P secured from the corpuscle samples of rabbit A and rabbit B.

The blood volume per kgm of rabbit weight is found to be 42 cc., per kgm of chick weight 45 cc.

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52. DETERMINATION OF THE RED CORPUSCLE CONTENT

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SOME time ago, a method of determination of the red corpuscle content was described (HAHN and HEVESY, 1940). Corpuscles containing labelled phosphorus compounds are introduced into the circulation of the rabbit, for example, and the labelled phosphatide content, or the labelled acid-soluble phosphorus content of the corpuscles of samples, secured after the lapse of few minutes, is compared with the corresponding content of the corpuscles injected. Such a comparison indicates to what extent the labelled corpuscles introduced into the circulation are diluted by the corpuscles of the circulation, and it permits thus the calculation of the red corpuscle volume of the rabbit.

In the present note, a simplified form of the above mentioned method is described. The modified method is based on the comparison of the total labelled phosphorus content of the corpuscles injected, with the total labelled phosphorus content of the corpuscles secured after the injection took place. The corpuscles are labelled *in vitro*. A blood sample of the rabbit is shaken in the thermostat for 1 to 2 hours in the presence of labelled sodium phosphate. By this procedure the corpuscles get labelled. The blood containing the labelled corpuscles is then reintroduced into the circulation of the rabbit. This modification of the previously described procedure was worked out in view of a possible clinical application of the method.

LABELLING OF THE CORPUSCLES

About 20 cm³ of blood are removed from the rabbit, placed in a flask the walls of which are coated with paraffin, labelled phosphate of negligible weight (see below) is added, and the blood is gently shaken in a thermostat for 2 hours at 37°. ⁽¹⁾ 10 cm³ of the blood are then reintroduced into the circulation of the rabbit. From the remaining blood, two standard samples, each of them weighing about 3 gm, are prepared.

⁽¹⁾ This temperature was chosen to accelerate the penetration of labelled phosphate into the corpuscles.

3 minutes after the injection of the labelled blood into the jugular vein, a few cc. of blood are taken from the carotis. Further samples are taken at later times. The heparinized blood samples are centrifuged, the corpuscles are weighed and brought into solution by wet ashing; subsequently, 80 mgm of sodium phosphate are added, and the phosphate content of the solution is precipitated as ammonium magnesium phosphate. The standard samples are treated in a similar way. In view of the much larger activity of the standard samples, only $1/20$ of the solution obtained after ashing the sample is precipitated.

Let us denote the amount of corpuscles injected into the rabbit by A, the ratio of the activity of 1 gm corpuscles of the blood injected and of the activity of 1 gm corpuscles secured from the circulation after the injection as B, then the total amount of the corpuscles present in the circulation (X) is given by

$$X = A \cdot B.$$

In some of our experiments, we used ^{32}P prepared from carbon disulfide which was previously irradiated by a neutron beam. Should some of the ^{32}P present in the solution obtained by the extraction process be adsorbed by colloidal particles or be present in the solution in another not properly dissolved state, this part of the ^{32}P will be found after centrifuging the standard blood sample in the fraction containing the erythrocytes, while in the circulation this part may be taken up by the reticulo-endothelial cells. In order to avoid a possible error due to such an effect, we did not add the ^{32}P directly to the blood to be investigated but to a small blood sample which was centrifuged at once. The plasma of the last mentioned sample containing ^{32}P was added to the blood to be shaken in the thermostat.

An alternative method is the following one. Labelled phosphate is administered to a rabbit, the blood of which thus becomes labelled. Few cc. of the labelled blood are introduced into the circulation of another rabbit after the lapse of several hours. By this procedure the activation *in vitro* can be avoided.

Is the Label of the Corpuscles Properly Conserved?

The method described above is based upon the assumption that the ^{32}P introduced into the corpuscles while the blood containing labelled phosphate is shaken in the thermostat, is not given off during the experiment. One might expect that after the introduction of active blood into the inactive circulation, ^{32}P will leave the corpuscles and get replaced by ^{31}P atoms of the plasma. Such a process, if taking place at a sufficient rate, would clearly frustrate the application of the method. Since, however, a mixing of the blood introduced into the circulation of the rabbit with the circulating blood does not last more than some minutes or less, the blood sample can be secured, for example 3, 5, 7, and 9 minutes after the injection took place.

The loss of activity of the corpuscles in experiments in which active corpuscles were shaken in the thermostat with inactive plasma for 12 min was found to amount to 1.5 per cent, only. (Cf. also HAHN and HEVESY 1940). That a possible loss of the labelling ^{32}P by the corpuscles does not influence our results is also seen in Table 1, in which

the average of the activity of 1 gm of corpuscles secured from 12 rabbits after 3, 5, 7, 9, and 15 min, respectively, is stated. The values obtained after 5, 7, and 9 min do not differ from each other within the error of experiments (± 5 per cent). The value obtained after the lapse of 15 min, possibly indicates a slight loss of ^{32}P by the labelled corpuscles.

The fact that, within a time amply sufficient to carry out determination of the corpuscle volume, the label of the corpuscles remains conserved is due partly to the comparatively slow rate of passage of the phosphate ions through the corpuscle wall, and partly to the fact that the easily renewable (activated) acid soluble P content of the corpuscles is much higher than the corresponding fraction of the plasma. The major part of the ^{32}P

TABLE 1. — ACTIVITY OF 1 GM CORPUSCLES SECURED FROM DIFFERENT RABBITS AT DIFFERENT TIMES AFTER THE INJECTION OF LABELLED BLOOD (CORPUSCLES)

Time in minutes	3	5	7	9	15	Rabbit
Activity (taking the activity found after the lapse of 3 minutes to be = 100)	100	106		104		B. 2
	100	102	95		96	B. 3
	100	104	91		89	B. 6
	100	105		106	97	B. 7
	100	107		105	92	B. 8
	100	95	96	110		B. 9
	100		102	99		B. 10
	100		100	96		B. 11
	100	93	95	95		C. 1
	100	100	97			C. 2
		100	97	93		C. 3
	100	104		95	-	C. 4
Average activity	1100 : 11 =100	1016 : 10 =102	773 : 8 = 97	903 : 9 = 100	374 : 4 = 94	

which entered the corpuscles while the blood was shaken in the thermostat is present as easily exchangeable organic acid soluble P. Now, the ^{32}P atoms have the same chance to leave the corpuscles as have the ^{31}P atoms present in the same state. The ratio of ^{32}P and ^{31}P atoms in the corpuscles is, however, much smaller than the corresponding ratio in the plasma. Correspondingly, the chance of a ^{32}P atom to leave the corpuscles is comparatively small. The corpuscles of 100 gm of rabbit blood contain about 20 mgm easily renewable acid soluble P atoms, while the plasma contains only about 2 mgm P atoms; the ^{32}P content of 1 gm corpuscles in the labelled blood injected is about the same as the ^{32}P content of 1 gm plasma. The ^{32}P atoms have thus a much greater chance to enter the corpuscles than to migrate in the opposite direction. Of course, if an exchange equilibrium of ^{32}P between plasma and corpuscles is reached, this statement is no longer valid, but — as follows from the

above data — after shaking the blood at 37° for 2 hours, the $^{32}\text{P} : ^{31}\text{P}$ ratio of the plasma is still about 10 times the corresponding ratio in the corpuscles.

Errors due to the Activity of the Plasma

We do not inject active corpuscles but active blood into the circulation of the rabbit. The plasma of the rabbit becoming thus active, some active phosphate will penetrate into the corpuscles in the course of the experiment, increasing thus the ^{32}P content of the corpuscles. Such a process may entail a source of error. If, besides the corpuscles injected, labelled corpuscles are formed in the circulation, the value calculated for the erythron from the dilution figures will clearly be found too low. In order to estimate the error due to the above mentioned process the following experiment was carried out. Active plasma is injected into the blood of a rabbit and blood samples are secured at different times. The corpuscles are separated and their activity is determined. The figures obtained (cf. Table 2) show what percentage of the plasma activity enters the corpuscles during the experiment (3 to 12 min), similar values being obtained in further experiments. In our red corpuscle determination, the activity of 1 gm corpuscles injected was about the same as the activity of 1 gm plasma injected, i. e. in the relative units of Table 2 = 1000. Therefore the figures of the 4th column of Table 2 give almost exactly the percentage error of the red corpuscle determination due to the penetration of ^{32}P from the plasma into the corpuscles in the circulation of the rabbit.

It is of interest to compare the rate of penetration of ^{32}P from the plasma into the corpuscles in experiments *in vitro* with the figures obtained in the above described experiment *in vivo*.

Experiment in vitro

Inactive blood was brought to 37° in the thermostat, active plasma of negligible weight was then added and the activity of the corpuscles was determined at different times. After the lapse of 3, 6 and 12 min, respectively, the corpuscles were found to contain 5.4%, 8.8%, and 12.9% of the activity of the plasma. These figures were corrected for the activity due to adhesion of active plasma to the corpuscles which was found to make out 2% of the plasma activity. In experiments *in vitro* in which the plasma activity does not much change during the experiment the activity penetrating into the corpuscles during 3 to 12 min is thus quite appreciable. In experiments *in vivo*, however, the plasma activity rapidly decreasing after the injection of the active blood, the amount of ^{32}P penetrating into the corpuscles is much smaller

(cf. Table 2). If the plasma activity would disappear with the same speed in experiments *in vitro* as in experiments *in vivo* we would obtain the figures seen in the 5th column of Table 2.

The following example shows how column 5 of Table 2 was calculated. Calculation of the value obtained after 2 min. The average activity of 1 gm plasma during the 3 first minutes can be estimated to be 500. As, after 3 min, in the experiment *in vitro* 1 gm corpuscles was found to contain 5.4% of the activity of 1 gm plasma, the figure registered in column 5 works out to be 2.7. The average activity between 3 and 6, and between 6 and 12 min, respectively, is estimated to be 200 and 130, respectively.

When comparing the activity of the corpuscles with the activity of the plasma, we can calculate the increase in activity of the corpuscles and compare this activity increase with the activity of the corpuscles which we inject in our usual experiments in which blood is shaken with active phosphate in the thermostat before the injection.

TABLE 2. — PENETRATION OF ^{32}P OF THE PLASMA INTO THE CORPUSCLES AFTER INJECTING LABELLED PLASMA INTO THE RABBIT

Time in min	Activity of 1 gm corpuscle	Activity of 1 gm plasma	Percentage of the plasma injected present in the corpuscles	
			found	calculated from experiments <i>in vitro</i>
0	0	1000	0	0
3	26	260	2.6	2.7
6	33	158	3.3	3.4
12	40	99	4.0	3.9

Adhesion of Active Plasma to the Corpuscles

The activity of the plasma may also influence the results obtained in another way as mentioned above. Centrifuging of blood does not lead to corpuscles entirely free of plasma. After centrifuging blood for 25 min, in a centrifuge making 5000 revolutions per minute, we find the corpuscles to contain 2% plasma (cf. also HAHN and HEVESY, 1942). In 1 gm corpuscles prepared from the blood to be injected, we shall therefore find only 0.98 gm corpuscles, the remainder being composed of plasma. In the blood to be injected, the activity of 1 gm corpuscles is about equal to the activity of 1 gm plasma, and the error due to the presence of plasma in the blood injected can thus be disregarded. However, other conditions prevail in the blood samples secured from the rabbits at different times. In the last mentioned samples, the adhering plasma is much less active

than the corpuscles to which the plasma adheres. The activity of 1 gm of the corpuscles secured from the active circulation is thus not strictly comparable with the activity of 1 gm of the corpuscles injected. Knowing the activity of the plasma at different times (cf. Table 2), we can correct for this discrepancy. The red corpuscle value calculated without taking regard to the above mentioned source of error will be 1.5%, 1.7% and 1.8%, respectively, too high. We could eliminate the above mentioned sources of error due to the activity of the plasma by removing the active plasma and replacing it by an inactive one. However, this step would complicate the procedure.

Not only, however, remains the error due to the adherence of the plasma to the centrifuged corpuscles within the errors of the experiment, it is in fact to a large extent compensated by the error due to the penetration of active phosphate from the plasma into the corpuscles during the few minutes which elapse between the injection of the active blood and the collection of the samples.

An estimate of the three errors of the experiment, — viz. (1) adhesion of plasma to the centrifuged corpuscles, (2) penetration of ^{32}P from the plasma into the corpuscles in the circulation of the rabbit (error due to the fact that we do not inject labelled corpuscles but labelled blood), (3) loss of ^{32}P by the corpuscles during the experiment — is seen in Table 3. After the lapse of 3 min., the plasma adhering to the corpuscles is thus less active (per gm) than the corpuscles to which it adheres. Since we determine the weight of the corpuscles + adhering plasma, and the adhering plasma (per gm) is only $\frac{1}{4}$ as active as the corpuscles, we find the activity of the corpuscles too low, the error being $2 - 2 \cdot \frac{1}{4} = 1.5$ per cent. Correspondingly, after 5 and 7 min, respectively, we commit an error of about 1.7 per cent.

TABLE 3. — ESTIMATE OF DIFFERENT ERRORS OF EXPERIMENT IN THE DETERMINATION OF THE RED CORPUSCLE VOLUME

Time in min	Percentage error due to adherence of the plasma to the corpuscles	Percentage error due to intrusion of ^{32}P of the plasma into the corpuscles	Percentage error due to the loss of ^{32}P by the corpuscles
3	+ 1.5	—2.7	+ 0.5
6	+ 1.6	—3.4	+ 1
12	+ 1.8	—3.9	+ 1.5

RESULTS

As seen in Table 4, the corpuscle content of the rabbit per kgm of fresh weight varied between 20.5 and 26.5 gm. It is difficult to decide whether an erythrocyte reserve present in the spleen and some other organs

interchanges with the erythrocytes of the circulating blood and, correspondingly, to what extent it participates in the dilution of the labelled corpuscles. Since our experiments were carried out with narcotized rabbits and, as shown by BARCROFT (1934), in ether narcosis the blood reserves of the body are to a very large extent released, our results are independent of the above mentioned source of error and indicate the total erythrocyte content of the rabbits investigated.

TABLE 4

R a b b i t	Weight in gm	Weight of corpuscles injected	Activity per gm corpuscles injected	Activity per gm corpuscle sample ⁽¹⁾	Total cor- puscle con- tent in gm	Corpuscle content in gm per kgm rabbit weight
B. 2	3050	3.40	2006	108	63.1	20.7
B. 3	2290	2.66	2470	140	46.9	20.5
B. 6	2470	3.98	1660	115	57.5	23.3
B. 7	2550	3.71	2160	153	52.4	20.6
B. 8	3200	3.38	2040	83.3	82.9	25.9
B. 9	1990	3.06	2450	181	41.4	20.8
B. 10	3160	3.10	2102	99.7	65.4	20.7
B. 11 ⁽²⁾	2010	3.23	2160	240	29.1	14.5

⁽¹⁾ Average of samples secured between 3 and 9 min (comp. Table 1).

⁽²⁾ Anemia following previous operation. Hematocrit value — 22.5.

Radio-Phosphorus and Radio-Iron as Indicators

HAHN, BALFOUR *et al.* (1941) determined the corpuscle volume of the dog, using radio-iron as an indicator. Within a few days after administration of radio-iron to dogs, most of the radio-iron present in the body is concentrated in the erythrocytes as a constituent of the hemoglobin molecules. Such erythrocytes labelled by the presence of radio-iron were used in the same way to determine the red corpuscle volume of the dogs as corpuscles labelled by the presence of radiophosphorus were applied by HAHN and HEVESY (1940) and by the present writers to determine the red corpuscle volume of the rabbit. In the determination of the red corpuscle volume, the radio-phosphorus method has the following advantages. Blood samples can be activated *in vitro*, while such a procedure cannot be carried out when using radio-iron. Corpuscles containing labelled haemoglobin can only be obtained in experiments *in vivo*. Furthermore, radio-phosphorus is very much easier to procure than radio-iron, since the preparation of radio-iron in sufficient quantities requires powerful tools in contrast to the preparation of radio-phosphorus.

Summary

A simplification of the method of determination of the red corpuscle content previously communicated is described.

A blood sample taken from a rabbit is shaken in the thermostat at 37° for about 2 hours in the presence of labelled sodium phosphate and, then, it is reintroduced into the circulation of the rabbit. A comparison of the total labelled phosphorus content (radioactivity) of the corpuscles injected with the total labelled phosphorus content of corpuscle samples secured, few minutes after the injection took place, leads to the value of the red corpuscles.

The red corpuscle content of the rabbits investigated varied between 20.5 and 26.5 gm per kgm of rabbit weight.

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P. F. HAHN, W. M. BALFOUR, J. F. ROSS, W. F. BALE and G. G. WHIPPLE (1941) *Science* **93**, 87.

53. THORIUM B LABELLED RED CORPUSCLES

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THE labelling of red corpuscles is an important application of radioactive indicators as labelled erythrocytes are applied among others in circulation studies and in the determination of blood corpuscle (blood) volume⁽¹⁾.

Labelling of red corpuscles with ^{32}P is made possible by the presence of comparatively large amounts of labile acid soluble phosphorus in the erythrocytes. Much of the ^{32}P which intrudes from the plasma into the red corpuscles speedily participates in the glycolytic and other enzymatic processes taking place in the erythrocytes. This participation involves incorporation into labile organic phosphorus compounds and thus "dilution" of the intruded ^{32}P . As a result of this "dilution" if in the course of 1 hour 1 mgm intruding phosphorus carries 1 μcurie into the corpuscles 1 mgm phosphorus moving from such corpuscles in the opposite direction carries into inactive plasma an appreciable smaller activity which amounts to about $1/_{12}$ or less of 1 μcurie only. Interchange of phosphate between plasma and red corpuscles is a continuous process not influenced by the presence of added labelled phosphate, the latter having a negligible weight. Injected into the human circulation, such labelled red corpuscles will lose 8 per cent or less of their activity in the course of 1 hour. In the course of 20 minutes the loss is certainly less than 3 per cent. This time interval is in most cases sufficient to carry out circulation velocity or blood volume measurements.

In a similar way, red corpuscles can be labelled by adding ^{42}K to a blood sample and shaking it in a thermostat for an hour or two⁽²⁾. In this case — due to the much higher potassium content of the erythrocytes — the intruded ^{42}K becomes "diluted" by endogenous potassium in a similar way as intruded ^{32}P gets "diluted" by endogenous phosphorus and correspondingly the ^{42}K labelled corpuscles injected into the circulation lose less than 4 per cent of their activity in the course of 1 hour.

⁽¹⁾ cf. G. NYLIN, *Svenska Vetenskapsakad. Arkiv Kemi A* 20, No. 17 (1945).

⁽²⁾ G. HEVESY and G. NYLIN, *Acta Physiol. Scand.* in print.

In some pathological cases with impaired circulation, mixing of the injected and circulating red corpuscles takes place at a much slower rate than under physiological conditions and accordingly it may be desirable to follow the path of the labelled erythrocytes in the circulation for many hours.

Such observations can not be carried out by making use of ^{32}P or ^{42}K labelled red corpuscles.

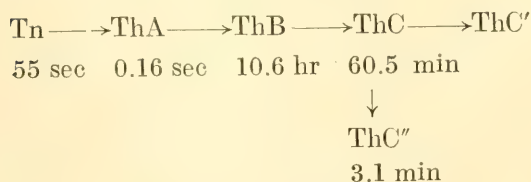
Recently ^{51}Cr was suggested as labelling agent of erythrocytes. Washed red corpuscles, labelled with $\text{Na}_2^{51}\text{CrO}_4$ *in vitro*, were injected intravenously into dogs and were found to retain their activity without significant loss to the plasma for approximately 24 hours. The previously determined red corpuscle volume could be re-determined within 5% for approximately 24 hr.⁽¹⁾

Neither β -particles nor positrons are emitted by ^{51}Cr but X-rays and γ rays of low intensity. These can be measured very conveniently by making use of a crystal counter which, however, requires expert assistance, in contrast to the running of a Geiger counter. Future progress in the measuring technique may eliminate the difficulties encountered today by a clinical institution, when trying to apply red corpuscles labelled with ^{51}Cr in corpuscle volume measurements.

In this note a labelling method of red corpuscles is described which permits to produce tagged erythrocytes which in the course of many hours lose only minute amounts of their radioactivity.

The method is based upon the introduction of thorium emanation (thoron) into the blood sample. The emanation, which has a half-time of 55 sec, penetrates speedily into the red corpuscles, decays inside them producing the active deposit of thorium which remains even for many hours to a very large extent fixed in the red corpuscles.

The sequence of the radioactive disintegration products of thorium emanation (Tn) is the following:



The ThA decays with a half-time 0.16 sec and can thus be disregarded. ThB, however, which has a half-time of 10.6 hr, comes soon in exchange equilibrium with the following disintegration products. Consequently the activity of the erythrocytes decays with the disintegration period

⁽¹⁾ S. J. GRAY and K. STERLING, 1950 (A. E. C. U. — 1072); K. STERLING and S. J. GRAY, 1950 (A. E. C. U. — 1026).

of ThB. This radioactive body emits soft β -rays, its disintegration products hard β -rays. One of these ThC'' emits, furthermore, very hard γ -rays. The approximate half-value thickness of the β -rays emitted by ThB and its disintegration products in aluminium is the following:

ThB (lead isotope) 1.6×10^{-1} mm

ThC (wismuth isotope) 1.2 mm

ThC'' (thallium isotope) 7.7×10^{-1} mm

The half value thickness of the γ -ray emitted by ThC'' is 17 cm.

As the β -rays emitted by ThC have almost the same hardness as those emitted by ^{32}P , they are very conveniently measurable.

In our experiment, a stream of oxygen after having brushed over the surface of a radiothorium preparation of about 1 mC activity was led through 4 ml of rabbit blood. Plasma and red corpuscles were then separated, washed 2 to 3 times with inactive plasma and then shaken with inactive plasma of the same rabbit for 2 hr at 37° in the thermostat.

Corpuscle and plasma samples were dried at 60° and 50 mgm of each sample placed in an aluminium dish; their β -activity was then determined with the Geiger counter. We determined the water content of each corpuscle and plasma sample and could thus calculate from the activity figures of the dry samples the activity of fresh plasma and fresh corpuscles.

The thorium emanation penetrates rapidly into the red corpuscles. It decays very rapidly as well and we soon find 1 gm of corpuscles to contain twice or more of thorium-active deposit than 1 gm of plasma. These figures depend on the velocity of the oxygen stream and on other experimental conditions. Through decay of thoron thorium B is formed in the plasma as well and a part of this thorium B is also taken up by the erythrocytes.

We found that after 2 hr shaking of active corpuscles with inactive plasma the corpuscles contained 50,560 counts but the plasma contained only 170. Thus the red corpuscles gave off only 0.34% of their activity in the course of 2 hr. The maximum loss was shown by a corpuscle sample containing 87,800 counts, with 403 counts in the corresponding plasma. In this case the loss was 0.46%.

All these activities were measured 24 hr after leading thoron into the blood, thus after more than 2 decay periods of ThB have already elapsed. Strong corpuscle activities can thus be obtained by leading thoron for a few minutes only through a blood sample.

That the erythrocytes so well retain their content of ThB and its disintegration products is partly due to the fact that much of the active deposit of thorium, present in the red corpuscles, is in a colloidal or protein

bound state. Much of the decay of thorium emanation leads to formation of such thorium B particles.

That much of the activity of the red corpuscles was due to colloidal or protein bound particles was shown by making use of the fact⁽¹⁾ that on a zinc plate, dipped into a solution containing colloidal ThB (lead isotope) or ThC (wismuth isotope), much more activity accumulates when acidifying the solution.

When 0.5 gm of corpuscles were hemolyzed by adding the same volume of water and one half of that volume was shaken after immersion of a zinc plate (2×2 mm) for 5 minutes, the washed zinc plate had an activity of 3540 counts per min. When first adding 0.5 ml of 1 N HCl to the other half of the hemolysate and then repeating the experiment, the zinc plate had an activity about 4 times as large, namely 14,897 counts per min than in the first mentioned experiment. The addition of acid converted much of the colloidal and protein bound active deposit into the ionic state. It follows that about $3/4$ of the active deposit present in the corpuscles was in a colloidal or protein bound state.

The results of blood volume determinations obtained by NYLIN and the present writer in a clinical investigation in which thorium B labelled red corpuscles were applied, will be shortly published.

Summary

By leading thoron (thorium emanation) through blood for a few minutes, red corpuscles labelled by the presence of the active deposit of thorium are obtained.

The red corpuscles of the rabbit conserve their activity for several hours, less than 0.5 per cent being lost when shaking the active erythrocytes with inactive plasma for 2 hr at 37° .

⁽¹⁾ G. HEVESY, *Sitzungsber. Wiener Akademie der Wiss.* **127**, Abt 2 a. (1918).

COMMENT TO PAPERS 51—53

In the first investigation (1939) on the labelling of red corpuscles with ^{32}P , labelled sodium orthophosphate was added to rabbits blood (paper 49). The penetration of ^{32}P into the red corpuscles was found to be a fairly slow process; most of the penetrated ^{32}P was, however, found to be present very shortly after penetration in the labile acid soluble phosphorus fractions of the erythrocytes. This distribution of the penetrated ^{32}P , in a pool of phosphorus compounds much larger than represented by the inorganic phosphate of corpuscles, make it possible to label red corpuscles fairly stably and apply these tagged erythrocytes in blood corpuscle volume determinations. If after incubation for 1—2 hr the inorganic phosphate of the red corpuscles alone took up substantial amounts of ^{32}P , after injecting such a sample into an inactive circulation, the ^{32}P would soon be lost. As the concentration of inorganic phosphate in the red corpuscles is lower than in the plasma, the exodus of ^{32}P would even take place in the inactive circulation at a more rapid rate than its incorporation in the course of its *in vitro* incubation. The participation of a large labile, and to some extent of a non-labile acid-soluble pool of phosphorus compounds in the uptake of ^{32}P , brings down the ^{32}P loss after injection of the labelled erythrocytes into inactive circulation to from one-twentieth to one-thirtieth of the loss which we would observe in the absence of such a pool. More recent investigations by GOURLEY (1952) and by GERLACH (1954) lead to the result that a part of the labelled acid-soluble phosphorus is already synthesized under participation of plasma phosphate in the course of the entrance of ^{32}P into the erythrocytes.

One day after subcutaneous injection of labelled phosphate to human subjects, the acid-soluble phosphorus of the corpuscles was found to contain almost twice as much ^{32}P than the plasma, while the plasma phosphatides contained only about one-third and the red corpuscle phosphatides, which are renewed at a slow rate, only about one-fortieth. These were the first experiments (1939) on the labelling of human erythrocytes (paper 49). *In vitro* experiments in which human blood was incubated with sodium phosphate containing ^{32}P were first carried out shortly afterwards by EISENMAN (1940). In the last mentioned experiments, a decrease of the incorporation of ^{32}P into the red corpuscles with decreasing temperature was observed. A very strong dependency of the rate of incorporation of ^{32}P into the erythrocytes of the rabbit was later ascertained (paper 50), the energy of activation of this process being found as high as 15,000 cal.

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First published in *Nature*, **134**, 879 (1934).

54. ELIMINATION OF WATER FROM THE HUMAN BODY

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SHORTLY after the first application of radioactive isotopes as indicators, the late H. J. G. MOSELEY and one of the present writers discussed the prospect opened by the introduction of this method, when indulging in a cup of tea at the Manchester Physics Laboratory. The latter then expressed the wish that an indicator might be found which would allow one to determine the fate of the individual water molecules contained in the cup of tea consumed. Even a man of the vision and outlook of the late H. J. G. MOSELEY considered this hope to be a highly utopian one.

The recent work of UREY and his collaborators brought, however, the above-mentioned wish within the range of realisation. Although diplogen and hydrogen, unlike the atoms of radioactive isotopes, are not practically inseparable by chemical means, yet if we add to a cup of tea a slight amount of heavy water and then find, for example, one per cent of the latter in the water which has left the body, we can assume that about one per cent of the 'normal' water molecules taken in with the cup of tea has shared the same fate.

That heavy water present in high dilution in the organism behaves like light water is borne out by the fact that the heavy water content of urine and other excreta is the same as that of ordinary tap water, within a limit of 1:100,000 as found by us and other experimenters.⁽¹⁾ If we slightly increase the heavy water content of the normal water we can assume that, with an accuracy sufficient for our purposes, the heavy water will show the same behaviour as the normal one. As a further argument in favour of this view, we may quote the results obtained when investigating the behaviour of highly diluted heavy water in the body of fishes.⁽²⁾

Our first step was to investigate, if water prepared from urine has the same density as the tap water drunk. The result was within 1:10⁶ in the affirmative. The preparation of water from urine was carried out by combined absorption and distillation processes. 55 samples of urine and other excreta were investigated and more than 1000 distillation

TABLE 1. — DENSITY OF WATER PREPARED FROM URINE
AFTER THE INTAKE OF DILUTED HEAVY WATER

Time elapsed since the intake of water started in hours	Urine (volume passed in cc.)	Density difference bet- ween water prepared from urine and 'normal' (dis- tilled) water
0.5	130	6×10^{-6}
0.8	190	10
1	230	15
1.2	210	21
1.5	230	23
1.8	290	25
2	160	21
2.5	80	20
4	120	18
8	130	20
10	290	18
17	320	20
23	140	19
24.5	210	18
42	820	19
67	1120	17
92	2100	17
244	—	10
340	—	8

processes carried out. One of us took then in one experiment 150 cc. and in another 250 cc. water containing 0.46 per cent heavy water showing a density difference against normal water of 480×10^{-6} . As the increase in density of the urine obtained after the intake of these quantities was only a few units in a million, an experiment was made in which 2000 cc. were taken. The increase in the density of the water obtained was then up to $25 : 10^6$. Some of the results are seen from Table 1.

From the above figures, it follows that after half an hour from the beginning of the intake of water, some of the water drunk is found in the urine, though only 0.2 per cent of the amount taken. The bulk of the water leaves the body at a slow rate and it takes 9 ± 1 days before half of the water taken has left the body.

We controlled the water balance during the experiments and found (in hot summer weather) that on an average 60 per cent of the water lost, left the body through transpiration and evaporation. In the possession of these data, and as we find that the density of urine water and transpiration water is the same within the limits of our accuracy relevant for these considerations (± 5 per cent of the density excess), we can calculate the time which elapses before half of the water taken left the

body by an independent method. The result works out again as 9 ± 1 days. By dividing the last figure by $\ln 2$, we get for the average time a water molecule spends in the body 13 ± 1.5 days. To explain this comparatively long time, we have to assume that most of the water taken becomes completely mixed with the water content of the body. This assumption can be tested by calculating the water content of the body of the experimenter from the amount of diluted heavy water taken, and the density of the water prepared from urine any day except the first one. We arrive at a water content of 43 ± 3 litres, namely, 63 ± 4 per cent in fair accordance with known data.

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COMMENT ON PAPER 54

SHORTLY after the first application of radioactive isotopes as indicators, in the spring of 1913, the late H. J. G. MOSELEY and the present writer discussed the prospect opened by the introduction of this method, when indulging in a cup of tea at the Manchester Physics Laboratory. The latter then expressed the wish that an indicator might be found which would allow us to determine the fate of the individual water molecules contained in the cup of tea consumed. Even a man of the vision and outlook of the late H. J. G. MOSELEY considered this hope to be a highly utopian one. It was the discovery of heavy water by UREY, which brought the above-mentioned wish within the range of realization. From the dilution figure of a known volume of administered labelled water, the water content of the organism was evaluated. This was the first application of isotopic tracers in clinical studies, and the first application of the device of isotope dilution in life sciences [paper 54 and HEVESY and HOFER, (1934) where a more detailed presentation of the results obtained is given]. At present, not heavy water but hyperheavy (tritiated) water is mostly used in such investigations. From the water content of the organism, conclusions can be drawn among others as to its fat content, as shown recently at the Donner Laboratory by PRENTICE *et al.* (1951). Assuming the lean body to contain 73 per cent of water, the fat content is calculated according to the formula: per cent body water = $0.73(100 - \text{per cent fat})$.

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55. EXCRETION OF PHOSPHORUS

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BECAUSE of the great importance of phosphorus in the formation of bones and the functional significance of a great variety of phosphorus compounds the balance of phosphorus intake and excretion has been investigated in numerous cases. A vast literature on this subject is available in which often also the route of secretion is considered, that is, the ratio in which the excreted phosphorus is to be found in the urine and faeces of the human subject or animal investigated. What percentage of the phosphorus excreted in the faeces is due to non-absorbed material and how much to phosphorus, originating from the body proper is, however, not yet known. Neither is any statement to be found on the fate of the individual phosphorus atoms, for example the phosphorus taken up with the food on one certain day. By using radioactive phosphorus as indicator we can follow the circulation of the phosphorus taken up at a certain date with food, the route it takes, and the rate at which it leaves the body. Some information on this subject has already been given.⁽¹⁾ In this paper we are communicating the results of investigations in which the excreta of human subjects, produced in the course of few months, were investigated both by radioactive and by chemical methods. Data are also given on the phosphorus excretion of rats.

GENERAL EXPERIENCE AS TO PHOSPHORUS EXCRETION

Ingested phosphates are excreted partly in the faeces and partly in the urine, the ordinary distribution in adult man being about two thirds in the urine and one third in the faeces. Conditions that diminish the solubility or promote the precipitation of phosphorus in the intestinal canal, tend to reduce the amount excreted in the urine and to increase

⁽¹⁾ O. CHIEWITZ and G. HEVESY, *Nature* **136**, 754 (1935); *Kgl. Danske Vidensk. Selsk. Biol. Medd.* **13**, 9 (1937); L. HAHN, G. HEVESY and E. LUNDSGAARD, *Biochem. J.* **31**, 1706 (1937); W. E. COHN and O. M. GREENBERG, *J. Biol. Chem.* **123**, 185 (1938).

that in the faeces. Vice versa, anything that favours solubility of phosphate in the alimentary tract, augments absorption and increases urinary phosphorus at the expense of the faeces. Thus, diets high in calcium and low in phosphorus lead to high faecal output and phosphorus deficiency, probably because the phosphate forms an insoluble precipitate of calcium phosphate in the intestine. It has often been observed that fatty acids, by diverting calcium from phosphoric acid, may release the latter for absorption. Anything which tends to produce a more acid medium in the intestine, exerts a favourable influence on the phosphorus absorption. Thus the ingestion of hydrochloric acid increases the urinary phosphorus at the expense of the faeces. The daily excretion of phosphate in the urine of an adult in normal conditions varies from 0.3 to 2 gm of P. A careful determination of the average daily phosphorus intake⁽¹⁾ of 25 college women has shown an intake of 1.40 gm, which is thus somewhat higher than required by the Sherman Standard (1.32 gm). In experiments⁽²⁾, in which the subjects used, were students and an acid-forming diet containing 780 gm, milk was administered, the daily phosphorus intake was found to be 1.98 gm. When as large an amount as 10.8 gm P was administered to a human subject, an output of 8.9 gm was found, 79% of the latter being present in the urine and 21 % in the faeces. About one fourth of the phosphate fed was stored⁽³⁾. In the early hours of the day the rate of excretion in the urine is minimal⁽⁴⁾, and then it rises during the course of the day, to reach a maximum at 4 or 5 in the afternoon. The level of excretion is then maintained for the remainder of the day and throughout the night. Within wide limits, there is no relationship between the amount of urinary phosphate and urinary volume. The rate of phosphate excretion is independent of the rate of water elimination even when, owing to copious diuresis, the urinary phosphate is below the level of the plasma phosphate⁽⁵⁾. As to the phosphorus excretion in animals, we wish only to mention the following data collected by us. Rats weighing about 230 gm excreted daily 28.7 mgm P; within 7 weeks the ratio urine P: faeces P varied between 1.3 and 2.4, the average being 1.6.

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EXCRETION EXPERIMENTS

The most suitable method of analysis of urine was found to be the following: Evaporate to dryness an aliquote, preliminarily treated with fuming nitric acid and determine its activity. Another smaller known fraction is digested in a Kjeldahl flask and its P content determined by the method of FISKE and SUBBAROW. The method tried first, based on the precipitation of ammonium magnesium phosphate from the urine, was found to be unsatisfactory, as activity measurements have shown that a part of the inorganic P present in the urine, remains in solution after precipitation with magnesia or the magnesium citrate reagents. In view of the low activity of many of the faeces samples, we had to work up several gm of dry faeces. It was too troublesome to dissolve such a comparatively large amount; we have therefore chosen the following procedure: The sample was first treated with nitric acid and then dried on a sand bath below 300°, to avoid loss of phosphorus. The activity of the substance was then determined. Another known part of the faeces sample, in most cases weighing only 30 mgm, was digested in a Kjeldahl flask and its P content determined by the colorimetric method.

TABLE 1

Time after administration of labelled P	Volume of urine in cc.	Specific activity of urine P (% of the labelled P administered found in 1 mgm P)
3 hours	130	0.0051
5 "	80	0.015
7 "	110	0.0109
10 "	156	0.0059
11 "	88	0.0052
22 "	348	0.0033
27 "	170	0.0038
34 "	140	0.0024
44 "	570	0.0017
3 days		0.0013
6 "		0.00056
8 "		0.00064
13 "	Daily average	0.00052
16 "	950	0.0005
26 "		0.0006
32 "		0.00027
43 "		0.00016

Excretion of labelled phosphorus through the kidneys

In the experiment described first, the urine of a 40 years old female patient suffering from diabetes was investigated. The labelled sodium phosphate of negligible weight was given with a glass of milk. We intended originally to investigate the excretion of the above mentioned patient after treatment with insulin as well. However, because the patient was soon discharged from the hospital, this investigation could not be carried out.

The average daily P excretion in the urine was found to be 950 mgm, the maximum of the specific activity of the urine P is reached after 5 hours (comp. Table 1). The rapid decrease of the specific activity of the urine P, in the later stage of the experiment, is due to the rapid decrease of the plasma P activity with time; the labelled phosphate ions of the plasma being replaced by unlabelled ones already present in the body, primarily in the skeleton and the muscles. The specific activity of urine P, which is derived from the plasma inorg. P, first increases with time, due to the increased absorption of the labelled P administered into the circulation. Besides, by interaction with tissue phosphate, the plasma inorganic phosphate also becomes "diluted" by unlabelled P absorbed into the circulation from the food taken. The latter will therefore also influence the specific activity of the urine P.

In the experiment recorded in Table 2, the urine of a 22 years old male subject was investigated. The subject took only a minimal amount of food and drink. His daily urine excretion amounted to 610 cc., only containing 660 mgm P.

TABLE 2

Time since administration of the labelled P in hours	Volume of urine in cc.	Specific activity of urine P
3	75	0.0158
5	52	0.0205
7	59	0.0103
9	38	0.0090
11	54	0.0063
18	180	0.0049
23	150	0.0035
25	46	0.0026
27	50	0.0027
30	115	0.0032
33	80	0.0026
39	145	0.0022
48	175	0.0015
51	110	0.00097

In the course of the first day 6.8%, in the course of the second day 3.3% of the labelled P was excreted through the kidneys. Higher figures were found in the experiment on p. 542.

In the experiment recorded in Table 3, we wanted to ascertain the amount of labelled P excreted after a very short time. The male subject, 23 years old, excreted daily 750 cc. urine containing 825 mgm P.

TABLE 3

Time since administration of the labelled P	Volume of urine in cc.	Specific activity of urine P
20 min.	90	0.00076
45 min.	16	0.008
18 hours	520	0.0073

After the lapse of 20 minutes, an easily detectable part of the labelled phosphorus, was thus found in the urine amounting to 0.1 per cent of the labelled P administered. From the rate of urine production by the subject in question, we can conclude that only about 10 cc. urine were produced in the course of the experiment, 80 cc. being already present at the beginning of the experiment in the bladder. By taking account of the latter, the specific activity of the P found in the urine formed within the first 20 minutes, work out to be 0.0068, i. e. each mgm P found in the urine, produced within the first 20 min after drinking the labelled sodium phosphate solution, contained 0.0068 per cent of the phosphorus atoms present in the latter.

TABLE 4

Time after administration of the labelled P in hours	Volume of urine in cc.	Specific activity of urine P
4	125	0.047
18.5	590	0.0109
25	100	0.0043
28	87	0.0037
30.5	70	0.0035
34	130	0.0028
35	57	0.0026
38	160	0.0032
45	150	0.0028
75	800	0.0018

The fourth male subject investigated, 50 years old, excreted daily 730 cc. urine containing 790 mgm P. The daily excretion was high, namely 21.1% per cent in the course of the first and 6.4 per cent in the course of the second day. The specific activities are seen in Table 4.

In another set of experiments 5 cc. of a physiological sodium chloride solution were injected into the veins of each of twelve human subjects. The solution contained 1 mgm P as sodium phosphate, and also ^{32}P showing an activity of 10^{-5} milliCurie. We carried out these experiments to find out if the percentage of the activity, excreted within the first 24 hours through the kidneys, varies much

TABLE 5a. — LABELLED P ADMINISTERED PER MOUTH. AGE OF FEMALE SUBJECT 28 YEARS, WEIGHT 65 KGM

Time after administration of labelled P in days	Total volume in liter	Total P content in gm	% of total activity administered, present in urine
0—15	14.98	7.787	10.3
15—39	25.25	14.400	5.3
39—52	12.98	8.239	3.6
52—76	25.58	9.093	1.5

Excretion of labelled P in the course of 76 days 20.8 per cent.

Total P excretion in urine 39.519 gm.

Daily P average excretion 0.520 gm.

TABLE 5*b*. — FAECES

Time after administration of labelled P in days	Amount of dry faeces in gm	Total P content in gm	% of total activity administered, present in faeces
0— 9	265.7	3.600	4.44
0—15	640.2	4.840	5.46
15—33	900.5	7.185	0.98
33—41	333.1	2.135	0.14
41—53	438.6	2.744	0.08

Excretion of labelled P in the course of 53 days 6.7 per cent.

Total P excretion in faeces 16.924 gm.

Daily average P excretion 0.320 gm.

TABLE 6*a*. — LABELLED P ADMINISTERED PER SUBCUTANEOUS INJECTION. AGE OF FEMALE SUBJECT 20 YEARS, WEIGHT 65 KGM AT THE BEGINNING OF THE EXPERIMENT AND 70 KGM AT THE END.

Time after administration of labelled P in days	Total volume in liter	Total P content in gm	% of total activity administered, present in urine
0—16	18.75	13.00	8.0
16—40	24.79	16.91	3.1
40—53	15.25	16.17	1.7
53—77	27.20	12.24	1.5

Excretion of labelled P in the course of 77 days 14.3 per cent.

Total P excretion in urine 52.32 gm.

Daily P excretion 0.680 gm.

TABLE 6*b*. — FAECES

Time after administration of labelled P in days	Amount of dry faeces in gm	Total P content in gm	% of total activity administered, present in faeces
0— 6	132.6	2.585	0.60
6—17	572.6	9.360	0.84
17—38	636.5	7.070	0.12
38—45	214.7	2.815	0.10

Excretion of labelled P in the course of 45 days 1.7 per cent..

Total P excretion in faeces 19.245 gm.

Daily P excretion 0.447 gm.

from individual to individual. Though the human subjects were kept on the same diet, both the total P content and also the activity excreted within the first 24 hours varied markedly from individual to individual, as seen in Table 7.

TABLE 7. — % OF LABELLED SODIUM PHOSPHATE, ADMINISTERED PER INTRAVENOUS INJECTION TO HUMAN SUBJECT, EXCRETED WITHIN THE FIRST 24 HOURS THROUGH THE KIDNEYS

Human subject	Weight in kg	Volume of urine in cc.	Total P in mgm	% of labelled P recovered	Specific activity of faeces P
A	75	425	630	14.5	0.023
B	75	1515	1370	20.0	0.015
C	50	716	792	19.0	0.024
D	66	926	864	12.4	0.015
E	73	850	994	23.0	0.023
F	56	833	373	9.9	0.027
G	62	790	839	8.0	0.096
H	71	1164	1061	8.5	0.0080
I	80	1100	561	4.0	0.0071
J	61	632	707	14.4	0.020
K	64	420	518	8.9	0.017
L	71	1405	703	12.3	0.018

Excretion of labelled phosphorus through the bowels and the kidneys

In the case of 2 female subjects, we investigated the excretion in urine and faeces over a period of several weeks. The results are seen in Tables 5a and 5b, resp. 6a and 6b.

In the first experiment (Table 5), the labelled phosphorus found in the faeces was partly non-absorbed P and partly such originating from the body proper. In the second experiment, registered in Tables 6a and 6b, the labelled P being not given by mouth, the labelled P present in the faeces must have originated solely from the body phosphorus and got through the digestive juices into the faeces. The lower total phosphorus excretion in the last mentioned case (Table 6), is presumably partly due to the remarkable increase in weight of the subject in question during the experiment.

Comparison of excretion through the bowels and the kidneys

From the labelled P administered by mouth, in the course of two months 6.7% were excreted in the faeces. When given by subcutaneous injection about 1.7% left through the bowels. The latter must have reached the intestinal tract with the digestive fluids. These carry labelled P just as well, when the latter was administered by mouth; we have, therefore, to assume that somewhat less than $\frac{1}{4}$ of the 6.7% labelled P found in the faeces originated from the body proper. The same ratio was found in our former experiments⁽¹⁾, while the absolute amount excreted in the course of the first week was in those cases 2.5 times as high as found in the present cases. The labelled phosphate which left the body unabsorbed was, therefore, 6.7%—1.7% — 5.0%. We will now turn our attention to the result

⁽¹⁾ O. CHIEWITZ and G. HEVESY, *loc. cit.*

of chemical analyses which indicate the excretion of total P contained in the diet of the subjects.

From the total P of the diet excreted 33% and 35% left the body through the bowels in two experiments, thus a decidedly higher figure than found for the excretion of the labelled sodium phosphate. It is also higher than found in a former case for the amount of labelled P which left the body absorbed (13%). To account for this discrepancy two different explanations can be put forward. According to one explanation, phosphorus present in some of the organic phosphorus compounds of the food, is less effectively resorbed than the labelled inorganic phosphorus added to the food. Such P is only split off in the lower region of the intestinal tract, in which place it has more chance to form insoluble calcium phosphate, for example, than in the more acid upper region. An alternative explanation is, that it is not the binding of the phosphorus in the compound which matters, but the mechanical protection of the phosphorus compounds present in the food. From solid undigested particles, the phosphorus particles will not be leached out properly. As to the resorption of phosphorus, in a recent work, carried out in VERZAR's laboratory, LASKOWSKI⁽¹⁾ has shown that the phosphate radical present in sodium glycerophosphate, introduced artificially into the upper part of the small intestine, splits off rapidly. The effect of this fast process is that the phosphate of the above mentioned compound is absorbed into the circulation as quickly as that of the sodium phosphate. When experimenting on rats an absorption of 68% of the P administered was ascertained, after the lapse of one hour, with either compound. In the case of sodium phytin 62%, in that of sodium diphosphoglycerinate only 42% of the P content was resorbed. When the phosphorus compounds were introduced into the lower part of the small intestine, the percentage absorbed into the circulation was much smaller⁽²⁾ and amounted, in the case of sodium phosphate, to 38% of that introduced. The difference observed, is presumably due partly to the greater activity of phosphatases in the upper part of the intestinal tract, partly to the greater acidity prevailing there. We mentioned already that low acidity is favourable to the formation of insoluble phosphorus compounds. In so far as some of the phosphorus compounds present in the food decompose or get leached out in lower parts of the intestine, the yield will be lower and this may explain the difference observed between the absorption of labelled sodium phosphate and the total phosphorus present in the diet of the human subjects in question. We have also to consider that a part of the phosphorus may be contained in undigested fractions of the diet taken, protected by mechanical obstruction from the leaching effect of the digestive juices. We can expect more information on these points by replacing the administration of labelled sodium phosphate by that of vegetables, grown on labelled soil and thus containing labelled phosphorus compounds. We can also feed labelled eggs, layed by hens to which labelled sodium phosphate was administered, or labelled meat. The tracing of to what extent the labelled P is absorbed from these foodstuffs is to be expected to supply us with important information as to their digestibility and seems to be a rational approach to the study of digestion, especially if foodstuffs containing other labelled elements beside phosphorus could be administered as well. We can, however, also obtain a knowledge as to the amount of unresorbed P present in the faeces by an easier method than that sketched above, a method which we will describe in the following.

(1) M. LASKOWSKY, *Biochem. Z.* **292**, 312, 1937.

(2) Comp. also F. VERZAR and H. WIRZ, *Biochem. Z.* **292**, 174 (1937).

Origin of faeces phosphorus

Let us assume that all phosphorus present in the food, is absorbed into the circulation. In this case, all labelled P found in the faeces must originate from the body proper. It is ultimately the plasma inorganic phosphorus which is responsible for the formation of the phosphorus compounds present in the digestive juices and, therefore, the specific activity (activity per mgm P) of the faeces P should, in the above mentioned case, be equal to that of the plasma P. The specific activity of the inorganic plasma P being equal to that of the urine P, we shall expect to find the specific activity of the faeces P to be equal to that of the urine P. If the above assumption does not hold and a part of the faeces P is unabsorbed, inactive P originating from the undigested food, the specific activity of the faeces P will be found in that case to be lower than that of the urine P. The ratio $\frac{\text{specific activity of faeces P}}{\text{specific activity of urine P}} \times 100$ gives the percentage of P present in the faeces which originates from the body proper. If the food P is, for example, quantitatively absorbed, then the above ratio will work out to be 100. It is clear that different objections can be raised against the above considerations. One may object on the grounds that the specific activity of the plasma P, after the active P was added to the food, will first increase and then decrease, its variation with the time being thus an intricate one. Another objection which can be raised is that the tissue P of the organs involved, will also participate in the formation of the phosphorus compounds present in the digestive juices. These objections will not, however, be valid if we, before comparing the specific activity of urine P and faeces P, wait a considerable time, after administering the labelled P, before collecting the urine and faeces samples; preferably, samples should be collected for several days. After the lapse of a considerable time, most P present in the different compounds of the organs responsible for the production of the digestive juices will be in exchange equilibrium with the plasma P, these showing thus the same specific activity⁽¹⁾. In Tables 5a and 5b the amount of P found in urine and faeces and also its total activity is stated, from which the specific activities could be evaluated. In view of the very long duration of the experiment in question and the comparatively low activities shown by many of the faeces samples, the accuracy

TABLE 8. — SPECIFIC ACTIVITY OF URINE P AND FAECES P OF A FEMALE SUBJECT 7 RESP. 8 DAYS AFTER ADMINISTRATION OF LABELLED SODIUM PHOSPHATE PER INTRAVENOUS INJECTION

Fraction	Number of counts	P content in mgm	Specific activity (% of the activity administered per mgm P)
Urine P	107.4	9.01	11.9
Urine P	118.9	9.80	12.1
Faeces P	53.9	18.60	2.9

⁽¹⁾ A possible source of error may be found in the different rates of decrease of the specific activity of the inorganic P, and of some forms of organic P present in the body (comp. G. HEVESY and A. H. W. ATEN, *Kgl. Danske Vidensk. Selsk. Biol. Medd.* **14**, 5, 35 (1939).

of these experiments did not suffice to carry out such a calculation. To enable us to determine with sufficient accuracy the ratio of the specific activity of the urine P and the faeces P, we administered labelled sodium phosphate having an activity of about $\frac{1}{1000}$ milliCurie to a female subject and investigated the urine and faeces collected after the lapse of 7 and 8 days resp. As the faeces, collected after the lapse of 8 days, actually accumulated in the bowels at a somewhat earlier date, it is advisable not to compare urine and faeces collected the same day, but to compare the faeces with the urine collected one day previously. The result of this experiment is seen in Table 8. The specific activity of the total faeces P is only 24% of that of the urine P, the faeces P must therefore to a large extent originate from non-absorbed food, which is the only source of non-active P. It follows from the above figure that 76% of the P present in the faeces of the human subject in question is non-absorbed P, the rest originating from the body proper. This is, however, not to be interpreted as indicating a phosphorus absorption of the food taken amounting to only 24%. When interpreting the above figure, we must take into account that the P excreted through the kidneys amounts to about twice of that lost through the bowels, and the sum of both values represents the total P present in the food, if we assume that the subject in question is in P balance. We then find that only 25% of the total P present in the food was not absorbed into the circulation.

TABLE 9. — SPECIFIC ACTIVITY OF URINE P AND
FAECES P OF A FEMALE SUBJECT 28 DAYS AFTER
ADMINISTRATION OF LABELLED SODIUM PHOSPHATE
BY SUBCUTANEOUS INJECTION

Fraction	Specific activity
Urine P	8.07
Urine P	8.10
Faeces P ⁽¹⁾	1.77

⁽¹⁾ 18% of the total P found in the faeces was residual P obtained after the removal of the acid-soluble P (mostly calcium phosphate) and the traces of phosphatide P present. The specific activities of the different P fractions differed only to a minor extent.

Through the courtesy of Dr. KJERULF-JENSEN, who is investigating the P metabolism of a human subject by making use of radioactive P, we could investigate the faeces P and the urine P, collected 28 days after administration of labelled P. The results are seen in Table 9. From the ratio of the specific activities it follows that 20% of the P percent in the faeces was of endogenous origin and that of the total P present in the food 27% was not absorbed into the circulation.

Excretion by rats

We determined also the ratio of the specific activities of the urine P and faeces P excreted by a rat to which labelled sodium phosphate was administered, by subcutaneous, injection, 98 days previously. The results are recorded in Table 10. The rate of the specific activities is 2.4, thus 59% of the P found in the faeces originates from non-absorbed food P. The faeces P making 57% of the total

excreted P, we can conclude that, from the total food P taken by the rat, 33% was unabsorbed⁽¹⁾. The daily diet of the rat contained about 30 mgm P.

Similar values for the ratio of the specific activity of the urine P and faeces P were obtained in experiments with other rats kept on the same diet. The values obtained were 2.29, 2.47, 2.61, and 3.10 respectively. The samples were collected 30, 98, 10 and 20 days respectively after the administration of labelled sodium phosphate.

TABLE 10. — SPECIFIC ACTIVITY OF URINE P AND FAECES P OF A RAT 98 DAYS AFTER ADMINISTRATION OF LABELLED SODIUM PHOSPHATE BY SUBCUTANEOUS INJECTION. WEIGHT OF RAT 208 GM

Fraction	Specific activity (% of the activity administered per mgm P)
Urine	0.39×10^{-2}
Faeces	0.16×10^{-2}

It is interesting to compare the figures with those obtained when labelled sodium phosphate is administered to the rat by mouth, the animal killed, and the activity of the total intestinal tract investigated after the lapse of 4 hours. Such determinations were carried out by several investigators. We found⁽²⁾, 4 hours after administering labelled sodium phosphate (having a P content of about 1 mgm) to a fasting rat, that the total digestive tract and its content contained 12.7% of the phosphate administered; thus more than 87.3% was absorbed. The latter figure represents the lower limit, since some of the active P present was actually absorbed and got subsequently with the digestive juices into the intestinal tract again and some active P present in the food exchanged with the tissue phosphate of the intestinal tract before the active P had an opportunity to be absorbed. Still higher figures for the labelled P absorbed into the circulation are recorded by ARTOM, SARZANA and SEGRÉ,⁽³⁾ namely 88—97.9%; the duration of their experiments was appreciably longer, it varied from 9 hours to 4 days. A smaller absorption was found by DOLS and JANSEN⁽⁴⁾; after the lapse of 8 hours, the stomach and small intestine alone are stated by them to have contained 4.1—15.4% of the labelled sodium phosphate administered. COHN and GREENBERG⁽⁵⁾ found that, in the course of 8 hours, only 60—70% of the labelled sodium phosphate administered was absorbed.

The above data inform us as to the lower limit of the rate of absorption of the sodium phosphate administered, which can materially differ from the rate of

(1) K. M. HENRY and S. K. KON, [*Biochem. J.* **33**, 173 (1939)] emphasized recently that a large part of the P present in the gut becomes fixed by intestinal bacteria and is thus no longer available to the host. Bacterial bodies account for about 40% of the dry weight of rat faeces and P is a more essential component of bacteria than Ca.

(2) G. HEVESY and O. REBBE, *Kgl. Danske Vidensk. Selsk. Biol. Medd.* (In print).

(3) C. ARTOM, G. SARZANA and E. SEGRÉ, *Arch. Int. Physiol.* **47**, 245 (1938).

(4) J. L. DOLS and B. C. P. JANSEN, *Koninklijke Akad. van Wetenschappen* **40**, No. 6, (1937).

(5) W. E. COHN and O. M. GREENBERG, *J. Biol. Chem.* **123**, 185 (1938).

absorption of the phosphorus contained in the food, as discussed on p. 543. We get, however, trustworthy information on the latter point by comparing the specific activity of the urine P and the faeces P. As already mentioned, this comparison is based on the assumption that the specific activity of the P contained in the urine is equal to that of the P present in the digestive juices. We tested this assumption in the following way: Labelled sodium phosphate was administered to a cat, the animal was sacrificed after 17 days fasting and the phosphorus contained in the last urine produced, and also in the sample removed from the small intestine, investigated. We found 1 mgm P contained in each sample to have, within the error of the experiment ($\pm 4\%$), the same activity. The latter amounted to 0.003% of the total activity administered.

Information on the amount of endogenous P present in the faeces were also obtained by determining the P content of the faecal output of fasting animals⁽¹⁾. The conditions prevailing in such experiments are, however, far from being physiological ones. The amount of the digestive juices, and thus also of the phosphorus secreted into the digestive tract, will much depend on the amount and quality of the food administered. When, for example, 50 gm oil and 300 mgm labelled P as sodiumphosphate were administered⁽²⁾ to a fasting dog and the total P content of the intestinal tract investigated after the lapse of 5 hours, the latter was composed to an extent of about 75% of P endogenous origin.

Summary

After the lapse of 20 minutes, a slight amount (about 0.01%) of the radioactive phosphorus atoms taken by mouth as sodium phosphate can be recorded in the urine of a human subject. In the course of the first day 4–12% of the amount taken was recordered. When administering the labelled phosphate by intravenous injection the figures varied between 4 and 23%.

Phosphorus contained in the normal diet is less efficiently absorbed than sodium phosphate. As much as about 30% of the former leaves through the bowels. To what extent the latter is unabsorbed (exogenous) P and to what extent it is endogenous, thus derived from the body proper, can be determined by comparing the specific activity of the faeces P with that of the urine P. Such a comparison leads to the result that, in the cases investigated, 70–80% of the phosphorus present in the faeces was non-absorbed food P.

Similar determinations were also carried out on the excreta of rats.

It is emphasized that important information on the digestibility of different foodstuffs could be obtained by administering foodstuffs containing labelled phosphorus and other labelled elements, for example of vegetables grown on a soil containing labelled phosphate.

⁽¹⁾ R. NICOLAYSEN, *Biochem. J.* **31**, 107 (1937).

⁽²⁾ G. HEVESY and E. LUNDSGAARD, *Nature* **140**, 275 (1937).

COMMENTS ON PAPER 55

THE balance of phosphorus intake and excretion has been investigated in numerous cases. A vast literature on this subject is available in which the route of secretion is often considered, i. e. the ratio of urinary to faecal phosphorus of the human subject or the animal investigated. To what percentage the phosphorus excreted in the faeces is due to unabsorbed material and to phosphorus originating from the body proper were, however, not known before the application of radioactive indicators. This application of ^{32}P is reported in paper 55. The supposition that the specific activity of urinary phosphorus parallels that of digestive secretion, an assumption on which the method applied is based, was tested later by KJERULF—JENSEN (1941). He was able to support the above supposition. Among others, he compared the specific activity of the total phosphorus in samples of bile-pancreatic juice with the specific activity of urinary phosphorus, and found that 1 week after administration of labelled phosphorus the specific activity of the total phosphorus in bile-pancreatic juice samples was already very near the value of urinary phosphorus. When interpreting the figures obtained for endogenous and exogenous phosphorus content in faeces, we must envisage the possibility that some phosphate interchange takes place through the intestine wall and that, correspondingly, the endogenous phosphorus present in the faeces may be partly secreted and partly interchanged phosphorus. The method outlined in paper 55, can be used to determine what percentage of almost any element present in the faeces is of endogenous origin.

Reference

K. KJERULF-JENSEN (1941) *Acta Physiol. Scand.* **3**, 1.

Originally published in *Acta Physiol. Scand.* **3**, 123 (1942).

56. POTASSIUM INTERCHANGE IN THE HUMAN BODY

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INTERCHANGE between the potassium present in the cells and the potassium present in the extracellular fluid of animals, can be determined by using radiopotassium (^{42}K) as an indicator. Two methods were applied. One method is based on the comparison of the ^{42}K content of the plasma (extracellular) potassium and the ^{42}K content of the tissue potassium. (HAHN *et al.*, 1939, 1941; FENN *et al.*, 1941. Comp. also JOSEPH *et al.*, 1939.)

The other method (HAHN *et al.*, 1939, 1941) is based on the measurement of the amount of labelled potassium which disappeared in the course of the experiment from the plasma. Since the amount of potassium present in the tissue cells is many times larger than the amount of potassium present in the extracellular space, a rapid interchange between plasma (extracellular) potassium and cellular potassium will soon lead to a strong depletion of the plasma ^{42}K content. The rate of disappearance of ^{42}K from the plasma (extracellular space) is, therefore, a very sensitive measure of the rate of interaction between plasma (extracellular) potassium and cellular potassium. This method has the disadvantage that assumptions have to be made concerning the potassium content of the tissue cells. The advantage of the method is its great simplicity. It suffices to analyse the plasma or, as we shall see below, the urine. In experiments on human subjects clearly the last mentioned alone can be applied.

EXPERIMENTAL PROCEDURE

The labelled potassium was prepared by bombarding 80 mgm KCl with a deuterium beam in the Copenhagen cyclotron. The activity obtained was 1/500 milliCurie. We are much indebted to Professor J. C. JACOBSEN and Mr. O. N. LASSEN for preparing the radiopotassium. The sample obtained was purified and dissolved in 20 cc. water. It was taken 5 hours after the last meal by mouth by a male subject weighing

70 kgm. Urine samples were collected at intervals, and the activity of the samples was determined. As the ratio of $\frac{^{42}\text{K}}{\text{total potassium}}$ can be assumed to be about the same in the plasma and the urine, the determination of the activity of the urine potassium informs us on the ^{42}K content of the plasma potassium at the time of the formation of the urine.

The urine samples were ashed below 400° and their activity was compared with the activity of a standard preparation. This was prepared by adding an aliquot part of the solution of labelled purified potassium chloride taken by mouth to non-active urine. The ash samples weighed about 300 mgm. They were placed under the Geiger counter in aluminium dishes of 1.2 cm diameter. The potassium content of the samples was kindly determined by Dr. L. HAHN, using SOHL and BENNET's method.

We shall first consider the case that, in the course of the experiment, no interchange takes place between the extracellular and the cellular potassium. Then, the total ^{42}K absorbed from the intestine should be present in the extracellular fluid of the body except for the ^{42}K excreted and the amount taken up by the corpuscles. The amount of labelled potassium excreted in the course of 24 hours was found to be about 7 per cent of the amount present in the body, while as was found in the case of the rabbit and the rat, the corpuscles can be assumed to contain about as much ^{42}K as is present in the extracellular fluid. Assuming the potassium content of the plasma to be 20 mgm per cent (WECHSELBAUM *et al.* 1940) and the extracellular space to be 19 litres (BRODIE *et al.* 1939; KALTREIDER *et al.* 1940) in a human subject weighing 70 kgm, the extracellular fluid can be estimated to contain 3800 mgm potassium. Since about $\frac{1}{2}$ of the ^{42}K administered should in the above case be present in the corpuscles, 1 mgm plasma (extracellular) potassium will contain about $\frac{46.5}{3800} = 0.012$ per cent of the amount of ^{42}K administered.

Let us now consider the other extreme case, viz. full interchange in the course of 24 hours between extracellular and cellular potassium. To estimate the percentage of ^{42}K administered which will be present in 1 mgm plasma (urine) potassium, we have to estimate the potassium content of the tissue of the human subject in question. The major part of tissue potassium is found in the muscles. The average potassium content of human muscles is stated (CUMING 1939; MANGUS and MYERS 1940) to be 330 mgm per 100 gm fresh weight. The weight of the muscles was estimated, following a suggestion of Dr. BRANDT REHBERG, from the amount of creatinine excreted through the kidneys in the course of 24 hours. Dr. REHBERG most kindly determined the creatinine content of the urine and found a daily excretion of 1300 mgm. Prior to these determinations, the subject was kept on a vegetarian diet. In the deter-

mination of the weight of the muscles from the daily creatinine excretion it is assumed that the creatinine excreted has been formed in the body from creatine and the daily conversion of creatine to creatinine amounts to about 1.32 per cent of the weight of the creatine. From these figures the creatine content of the body can be estimated to be 99 gm. Assuming the creatine of the body to be located in the muscles and the creatine content of the muscles to amount to 0.39 per cent of the weight of the muscles, we arrive at the result that the weight of the muscles amounts to 25 kgm and that, correspondingly, the muscles contain about 82 gm potassium.

About $\frac{3}{4}$ of the potassium content of the mammalian body is found in the muscles. Assuming this to be the case in the human body, we arrive at the estimate that the body of the human subject in question contains 110 gm potassium of which 106 gm are located in the cells. The amount of extracellular potassium of the body is thus 3.6 per cent of the total potassium content of the organism. In the case of total interchange between extracellular and cellular potassium, only 3.6 per cent of the amount of labelled potassium absorbed into the circulation minus the ^{42}K excreted⁽¹⁾ should be present in the extracellular space. In the case of total interchange, 1 mgm urine potassium should thus, after the lapse of 48 hours, contain only 0.0010 per cent of the labelled potassium administered.

RESULTS

In the first experiment, the results of which are seen in Table 1, urine was collected during the first 2 days after drinking the solution containing the labelled potassium chloride. The presence of easily demonstrable amounts of ^{42}K could be shown as early as 12 minutes after drinking the active solution, while a negative result was obtained after the lapse of 5 minutes. The total urine was found to contain 10.5 per cent of the ^{42}K administered, the potassium content amounting to 3.4 gm. Thus, 1 mgm average potassium present in the urine contained 0.003 per cent of the ^{42}K administered. This is much less than the amount which was to be expected (0.012) assuming an absence of interaction between cellular and extracellular potassium (see p. 554).

The results of this preliminary experiment thus lead to the conclusion that a very substantial part of the ^{42}K absorbed into the circulation, must have found its way into the tissue cells while a substantially equal number

⁽¹⁾ We found the excretion of ^{42}K through the bowels to make out roughly 15 per cent of the amount excreted through the kidneys, the total excretion of ^{42}K in the course of the experiment being about $11 + 0.15 \times 11.13 =$ per cent.

TABLE 1. — EXCRETION OF LABELLED POTASSIUM
ADMINISTERED BY MOUTH

T i m e		Percentage of ^{42}K recovered in the urine
12	min.	0.0053
30	„	0.048
3	hours	0.46
5 $\frac{1}{4}$	„	0.65
16 $\frac{1}{4}$	„	4.75
40 $\frac{1}{2}$	„	3.00
48 $\frac{1}{4}$	„	1.56
Total		10.47
		Volume of urine = 2.041 cc.

of non-labelled potassium atoms migrated from the cells into the extra-cellular space.

In the two following experiments, besides determining the percentage of ^{42}K administered which was excreted in the course of 65 $\frac{1}{2}$ hours and the potassium content of the urine, urine samples were collected on three consecutive days between 9 and 11 $\frac{1}{2}$ hours and their activity and potassium content were determined. The results of these experiments are seen in Tables 2 and 3. While no pronounced difference in the specific activity of the potassium collected after 15, 39 and 63 hours is observed, in the

TABLE 2.— ^{42}K CONTENT OF URINE SAMPLES

Samples collected between (reckoned from the start of the experiment)	Potassium content per cc. urine in mgm	Percentage of ^{42}K administered present in 1 mgm urine potassium	
		Found	Calculated assuming total interchange
15 and 17 $\frac{1}{2}$ hours	3.77	0.0014	0.0010
39 and 41 $\frac{1}{2}$ hours	3.85	0.0011	0.0010
63 and 65 $\frac{1}{2}$ hours	3.05	0.0014	0.0010
0 and 65 $\frac{1}{2}$ hours	1.75	0.0024	

Total urine volume = 2.990 cc.

Percentage of the ^{42}K administered present in the total urine = 12.7 per cent.

early phases of the experiment potassium of higher specific activity was excreted than in the later phases. This follows from the higher value found for the specific activity of the average potassium present in the urine collected.

1 cc. urine collected between 9 a. m. and 11 $\frac{1}{2}$ a. m. was found to have an appreciably higher potassium content than 1 cc. of average

urine. This result is in accordance with the general experience according to which urine collected between the early hours of the day and noon has the highest potassium concentration.

TABLE 3

Samples collected between (reckoned from the start of the experiment)	Potassium content per cc. urine in mgm	Percentage of ^{42}K administered present in 1 mgm urine potassium	
		Found	Calculated
15 and 17½ hours	2.24	0.0015	0.0010
39 and 41½ hours	2.70	0.0011	0.0010
63 and 65½ hours	2.30	0.0013	0.0010
0 and 65½ hours	1.26	0.0025	0.001

Total urine volume = 2.880 cc.

Percentage of the ^{42}K administered present in the total urine = 9.1 per cent.

DISCUSSION

While the accuracy of the method applied does not suffice to determine whether a full interchange between the potassium of the cells and the potassium of the extracellular fluid took place, the results obtained clearly indicate (see the 2 last columns of Tables 2 and 3) that the greatest part of the labelled potassium ions and, thus, the greatest part of all potassium ions taken with the food find their way within 16 hours or less into the tissue cells while potassium ions formerly located in the cells move simultaneously into the extracellular fluid. In the average urine sample collected during the experiment, the potassium was found to be markedly less active than in the samples collected after the lapse of 15 hours. This is due to a higher activity of the potassium excreted in the first phase of the experiment. This result indicates that the interchange between cellular and extracellular potassium is not a very rapid process since it takes several hours before a large part of the extracellular potassium interchanges with the potassium of the cells.

Apparent Volume of Distribution

In connection with the experiments reported in this note, it is of interest to recall experiments by BOURDILLON (1937) in which the apparent volume of distribution of potassium chloride taken by him by mouth was investigated. The apparent volume of distribution =

$$= \frac{\text{amount absorbed into the circulation minus amount excreted}}{\text{increase in concentration in serum water}}.$$

If the potassium taken by mouth would remain in the extracellular fluid, the apparent volume of distribution should be 25 to 30 per cent of the body weight. BOURDILLON found, in experiments on himself, an apparent volume of exogenous potassium corresponding to 75 per cent of the body weight and a similar result was obtained in experiments on dogs by WINKLER and SMITH (1938) and on cats by FENN (1939). The apparent volume of distribution of the labelled potassium taken by mouth in the experiments described in this note, can be computed to be about 400 litres or 570 per cent of the body weight.

The striking difference in the results of BOURDILLON and the results arrived at in this note illustrates the great difference between accumulation and interchange of potassium. In BOURDILLON's experiment, additional potassium had to accumulate in some or all tissue cells and, if we assume the concentration of the extracellular fluid to remain isotonic, such an accumulation necessitates the exodus of other cations from the cells. This can only take place on a very restricted scale. Not so the interchange of extracellular potassium with cellular potassium. No other elements have to leave the cells to make place for the labelled potassium; it suffices that non-labelled potassium atoms make place for labelled ones.

Excretion of Sodium

The sodium content of the extracellular fluid is about 15 times larger than its potassium content. Since the potassium content of the urine does not differ much from its sodium content, we should expect a much

TABLE 4. — EXCRETION OF ^{24}Na ADMINISTERED THROUGH THE KIDNEYS

Urine sample secured after	Volume in cc.	Percentage of the ^{24}Na administered present in the urine sample
10 min.	5	0.00046
20 min.	2.9	0.0076
46 min.	3.9	0.036
98 min.	10	0.056
6 hours	80	0.31
19 hours	590	2.86
43 hours	1700	4.75
67 hours	1440	4.5
Total	3832	12.5

larger part of the potassium present at any moment in the extracellular space to be excreted through the kidneys in the course of the first 24 hours, for example, than of the sodium simultaneously present in the extracellular fluid. Thus, in the urine, a much larger part of ^{42}K administered than of ^{24}Na administered should be present. This is, however, not the case. As seen in Table 4, the percentage of daily excreted ^{42}Na does not much differ from the percentage of daily excreted ^{24}K . From this result it follows that most potassium ions present in the urine are such as were previously located in the cellular and not in the extracellular fluid; a conclusion which is in accordance with the result arrived at in the previous section.

RESULTS

Labelled potassium chloride was administered to a human subject and the potassium and the ^{42}K content of the urine samples collected within 65 hours were determined. After the lapse of 12 minutes, 5×10^{-5} part of the ^{42}K administered was found to be present in the urine. In the course of 48 hours, about 10 per cent were excreted through the kidneys.

Making the assumption that the ^{42}K content of 1 mgm potassium present in the urine is about the same as the ^{42}K content of 1 mgm potassium present in the plasma (extracellular space), from the ^{42}K content of the urine potassium the ^{42}K content of the extracellular fluid of the body can be computed. By this method, it was found that a very large part of the ^{42}K absorbed into the circulation found its way into the tissue cells in the course of 16 hours or less.

While the accuracy of the method does not suffice to determine whether a full interchange between the potassium of the cells and the potassium of the extracellular fluid took place in the course of the experiment, it is clearly shown that a very substantial part of the labelled potassium and thus of all potassium taken with the food, interchanges with the potassium located in the tissue cells in the course of some hours.

Labelled sodium was found to leave the body at a similar rate as labelled potassium.

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57. THE RED CORPUSCLE CONTENT OF THE CIRCULATING BLOOD DETERMINED BY LABELLING THE ERYTHROCYTES WITH RADIO-PHOSPHORUS

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THE red corpuscle content of the circulating blood is usually calculated from the plasma volume determined by the dye method and the hematocrit figure. Some time ago (HAHN and HEVESY, 1940; HEVESY and ZERAHN, 1942), the red corpuscle content of the rabbit was determined by making use of corpuscles labelled with radiophosphorus. This method was used by us to determine the erythrocyte content of the circulating blood of human subjects. Simultaneously, the corpuscle content was determined by the CO method and, furthermore, in some experiments the plasma volume was determined by the dye method.

EXPERIMENTAL

About 25 cm³ blood is taken by venous puncture. After adding heparin and about 1 cm³ plasma⁽¹⁾ of the same subject which contains a minute amount of labelled sodium phosphate showing an activity of about 1 μ Curie, the blood is placed in a glass bottle with paraffine coated walls and is gently rotated in a thermostat at 37° for 2 hours. After the lapse of this time, the radio-phosphorus added to the blood is found to be about equally distributed between the corpuscles and the plasma. A minor part of the labelled blood is kept as a standard preparation, while the rest is reintroduced into the human subject. The labelled corpuscles introduced into the circulation get soon mixed with all the circulating corpuscles and, as a result of this mixing, the activity of 1 gm corpuscles secured will be much lower than the activity of 1 gm corpuscles introduced. If we reintroduce 1 gm labelled corpuscles containing 1000 activity units while the circulation contains 1000 gm corpuscles, 1 gm corpuscles secured after the mixing, i. e. after the lapse of few minutes, will show an activity of 1 unit.

⁽¹⁾ The plasma is obtained by centrifuging blood containing labelled phosphate.

If we wish to determine the amount of circulating corpuscles we have to know (*a*) the amount of the injected labelled corpuscles, (*b*) the activity of 1 gm of these corpuscles, and (*c*) the activity of 1 gm corpuscles secured from the circulation after mixing. (*a*) The amount of injected corpuscles is obtained from the volume of the injected blood, the specific weight of the corpuscles (1.08), and the hematocrit value. (*b*) The activity of 1 gm of these corpuscles is found from measuring the activity of 1 gm corpuscles of our standard preparation mentioned above. (*c*) The activity of 1 gm corpuscles secured from the circulation after mixing is found when measuring the activity of 1 gm corpuscles secured 5–10 minutes after the injection.

Let us denote the amount of corpuscles injected into the circulation by *A*, the ratio of the activity of 1 gm corpuscles of the injected blood and of the activity of 1 gm corpuscles secured from the circulation after the injection by *B*, then the total amount of the corpuscles present in the circulation (*X*) is given by

$$X = A \cdot B$$

The blood sample is secured in the interval between 5 and 10 minutes, preferably after the lapse of 5 minutes, following the injection. 3 minutes after the injection, a mixing of the reinjected labelled blood with the non-labelled one might possibly not have occurred. On the other hand, the loss of activity by the corpuscles might become noticeable after the lapse of 10 minutes. Table 1 illustrates the above statement.

TABLE 1

Time in min	Per cent of corpuscles injected, present in 1 gm corpuscles
1.25	0.026
2.0	0.044
3.0	0.052
4.8	0.053
6.9	0.050
9.9	0.052
15.8	0.048
18	0.045
30	0.044

Sources of error

Three main sources of error have to be considered. (*A*) The centrifuged corpuscles are not free from plasma. (*B*) We do not inject active corpuscles, but active blood, and some radio-phosphorus may enter the corpuscles during the interval between the injection of labelled blood and the

securing of the blood samples. (C) During the last mentioned interval, some radio-phosphorus may leave the corpuscles.

(A) Though the amount of plasma adherent to 1 gm corpuscles of the standard preparation can be assumed to be equal to the amount of plasma adherent to 1 gm corpuscles secured after the injection, the incomplete separation of corpuscles and plasma involves an error which becomes obvious from the following example. 1 gm standard preparation is composed of 0.97 gm corpuscles and 0.03 gm plasma and has an activity = 1000. As the activity of 1 gm corpuscles is about equal to the activity of 1 gm plasma after 2 hours of rotation in the thermostat, 970 activity units are due to the corpuscles and 30 to the plasma. In a sample secured 3 minutes after the injection, the activity of 1 gm plasma declines to $\frac{1}{4}$ of its previous value. If, now, the activity of 1 gm of the sample is again assumed to be 1000, the share of the adherent plasma activity is only 8, the corpuscle activity being 992. When comparing the activity of the two corpuscle samples, we get thus a value of the corpuscle content of the circulation which is 2.3 per cent too high. The activity of the plasma adherent to the sample secured after the lapse of 10 minutes is still lower than the activity of the plasma secured after 3 minutes, *viz.* about $\frac{1}{10}$ of the activity of the plasma injected. When securing the blood samples after the lapse of 10 minutes, we overestimate the total corpuscle content of the circulation with about 3 per cent. The above result was obtained after centrifuging the blood sample with 5000 revolutions per minute for 10 minutes.

(B) As the injected blood contains active plasma, some radio-phosphorus will penetrate into the corpuscles during the experiment lasting 5–10 minutes. Phosphate penetrates at a much higher rate through the capillary wall (HAHN and HEVESY, 1941) than through the membrane of the erythrocytes. The bulk of the radio-phosphorus content of the plasma will, therefore, leave the plasma in the course of 5 minutes, much reducing the amount of radio-phosphorus which otherwise would penetrate into the corpuscles. Table 2 shows the rate of disappearance of radio-phosphorus from the plasma and the percentage of plasma

TABLE 2. — PENETRATION OF ^{32}P FROM THE PLASMA INTO THE CORPUSCLES AFTER INJECTING LABELLED PLASMA INTO THE CIRCULATION

Time in min	Activity of 1 gm corpuscle	Activity of 1 gm plasma	Percentage of the ^{32}P content of the plasma injected, present in the corpuscles
0	0	100	0
2	3.0	35	3
9	5.0	15	5
25	6.2	10	6

activity which penetrates into the corpuscles in the course of 25 minutes. The penetration of radio-phosphorus in the corpuscles during the experiment increases the activity of the corpuscles and, thus, makes the total corpuscle content of the circulation appear too low. The error in an experiment lasting 10 minutes amounts to 5 per cent.

(C) 1.5 per cent of the radio-phosphorus content of the corpuscles was found to be replaced by inactive phosphorus from the plasma in the course of 10 minutes. This loss ascertained in experiments *in vitro* makes the dilution figure and, thus, the corpuscle content of the body appear too high. That the loss of radio-phosphorus by corpuscles in the course of 10 minutes is markedly restricted, is not due exclusively to the fact that the phosphate ions penetrate only at a moderate rate through the corpuscle membrane, but also to the following. 1 gm corpuscles contains about $\frac{2}{3}$ as much inorganic P as 1 gm plasma and, moreover, comparatively large amounts of readily exchangeable organic P present in adenosintriphosphate and also in hexosemonophosphate, and some other acid soluble organic P compounds. The concentration of such readily exchangeable P atoms in the corpuscles is with an order of magnitude larger than the concentration of inorganic phosphorus. As soon as the active P atoms enter the corpuscles, they interchange with phosphorus atoms present in the organic compounds. After 2 hours shaking of the blood in the presence of labelled phosphate, a large part of radioactive P atoms present in the corpuscles will, thus, be found in the organic fraction and, consequently, the activity of the inorganic P of the corpuscles will be kept at an appreciably lower level than the activity of the inorganic P of the plasma.

An estimate of the different experimental errors in the determination of the red corpuscle content is seen in Table 3. The data of this table reveal that the value of the red corpuscle content obtained in experiments lasting 10 minutes is 0.5 per cent too low, the error being smaller in experiments of shorter duration.

Plasma volume determination by the means of the dye method

In a number of cases, the plasma volume was determined by means of the dye method described by GIBSON and EVELYN (1938). In this

TABLE 3. — ESTIMATE OF DIFFERENT ERRORS OF EXPERIMENT IN THE DETERMINATION OF THE ERYTHRON

Time in min	Percentage error due to adherence of the plasma to the corpuscles	Percentage error due to intrusion of ^{32}P of the plasma into the corpuscles	Percentage error due to the loss of ^{32}P by the corpuscles
10	+3	—5	+ 1.5

method, use is made of the blue dye T 1824, 10 mgm of which are dissolved in 5 cm³ of water.

Before injecting the dye, a blood sample which is used to determine the hematocrit figure is secured by venous puncture. Through the same cannula with which the blood sample is taken, T 1824 solution from a calibrated syringe is injected into the circulation. In order to remove the last traces of the dye present, the syringe is filled with blood which is also injected. This process is repeated three times.

Blood samples of the patient are drawn 15, 30, 45, and 60 minutes, respectively, after injection of the dye. After the blood sample has coagulated, the serum is centrifuged off and its dye content is determined by making use of a photoelectric colorimeter. The light absorption by the dye-free serum is previously ascertained in the same way.

The dye content of the different sera secured from the same patient at different times is plotted against time and, from the curve obtained, the dye content present at zero time is extrapolated. This procedure is necessary because it lasts some minutes until dye and blood are properly mixed and, during this time, some dye leaves the plasma. 15–30 minutes after injection, some dye was found to be present in the lymph of the *ductus thoracicus* (CARDOZO, 1941), and KÖSTER observed some dye in the bile 30 minutes after administration.

From the plasma volume obtained by means of the dye method and the hematocrit figure, the corpuscle content and the blood volume were calculated. The results are shown in Tables 6 and 7.

Determination of the corpuscle volume carried out with the CO method

We determined, furthermore, the corpuscle content applying the CO method. Carbon monoxide is prepared under the action of concentrated sulphuric acid on sodium formiate. The CO obtained is led through a sodium hydroxide solution, in order to remove any carbon dioxide or sulphuric acid spray possibly present. The purified CO is stored in a gasometer constructed by connecting two flasks containing diluted sodium hydroxide. Before filling the gasometer with CO, a stream of carbon monoxide is led through the liquid for some time, so that all air is removed from the gasometer. The gas stored in the gasometer was found to contain 97 per cent CO.

The CO is administered to the patients by a Krogh "basal metabolism apparatus" containing a known volume of CO (200 cm³) and a few litres of oxygen. For the transfer of the CO from the gasometer to the Krogh apparatus, use is made of a Luers glass syringe carefully kept at room temperature, the CO being injected in the tube leading from the oxygen flask to the spirometer and the tube washed with an oxygen current. The patient inhales the mixture of CO + O₂ in the course of

about 15 minutes. During this time, the oxygen taken up by the patient is replaced in the gaseous mixture. Subsequently, a blood sample is secured.

The determination of CO in the blood sample was carried out by making use of WENNESLAND's palladium chloride method (1940). Under the action of sulphuric acid, CO is given off by the blood sample placed in a flask. The CO released diffuses into another flask connected with the first one and containing a known amount of palladium chloride. Special precautions are taken to avoid a loss of CO while connecting the flasks. Some palladium chloride is reduced to palladium under the action of CO. When determining the amount of palladium chloride still present at the end of the experiment, we can calculate the amount of CO given off by the blood sample. The determination of the remaining amount of palladium chloride was carried out after rotating the blood-sulphuric acid mixture for 3—4 hours.

The blood volume was calculated according to the formula

$$\text{Blood volume} = \frac{\text{cm}^3 \text{ CO administered} \cdot 100}{\text{volume per cent CO in the blood sample}}$$

RESULTS

The results from determinations of the corpuscle content by the ^{32}P method performed on the same subject at different dates is seen in Table 4, while in Table 5 is given a survey of all our determinations carried out with application of the ^{32}P method. The mean value of the corpuscle content per kgm body weight is found to be 36.0 gm.

The corpuscle content obtained when applying the CO method is obvious from Table 6 which contains also data found when using the dye method. While the CO method is a direct method of determination of the corpuscle content, the dye method is an indirect one, the corpuscle content being calculated from the plasma volume and the hematocrit value.

As seen in Table 6, the corpuscle content determined when applying the CO method is larger than the corpuscle content found when making use of the ^{32}P method. This discrepancy is possibly due to the uptake of CO by other compounds than by the haemoglobin present in the corpuscles of the circulating blood (comp. ASMUSSEN 1942).

That the corpuscle content calculated from the plasma (dye) volume and the hematocrit value is larger than the corpuscle content determined when applying the ^{32}P method, can be interpreted in two different ways. (a) The plasma volume technique gives a falsely high plasma volume, (b) the plasma ratio of the circulating blood is lower than the plasma

ratio determined by the hematocrit reading of blood samples drawn from the body.

Some dye is lost by the plasma during the experiment. However, this loss is taken into account by extrapolating the dilution values obtained at different times to zero time. These considerations make it improbable that the above mentioned discrepancy is due to an overestimation of the plasma content by the dye method and suggest the alternative denoted by (*b*) to explain the discrepancy.

TABLE 4. — CORPUSCLE CONTENT OF
THE SAME SUBJECT DETERMINED AT
DIFFERENT DATES

No.	Date	gm corpuscle content
2	13/10	2370
4	22/10	2190
5	27/10	2930
6	3/11	2600
7	6/11	1940
9	13/11	1910
11	20/11	1920
14	27/11	1920
8	10/11	1940
10	16/11	1890
17	4/12	1940
12	23/11	2340
15	30/11	2100
20	11/12	2140
13	25/11	2280
16	2/12	2100
19	9/12	2090
18	7/12	2930
21	14/12	2810
22	21/12	2640

Already some years ago, it was suggested by SMITH, ARNOLD and WHIPPLE (1921) that the hematocrit reading does not give the true corpuscle-plasma ratio of the blood in the whole body. They found the red corpuscle volume determined by the CO method and the Welker method to be approximately 25 per cent lower than the red corpuscle

volume calculated from the plasma volume and the hematocrit reading and they interpreted their result as an indication of their suggestion. Furthermore, HOOPER, SMITH and WHIPPLE (1920) have shown that, after having lowered the hematocrit reading by haemorrhage, the measured red corpuscle volume (from the plasma volume and the hematocrit reading) did not agree with the red corpuscle volume predicted on the basis of the volume of the erythrocytes removed. If the red corpuscle volume before bleeding is equal to the red corpuscle volume after haemor-

TABLE 5. — CORPUSCLE CONTENT OF HUMAN SUBJECTS DETERMINED BY MAKING USE OF ^{32}P AS AN INDICATOR

No.	Corpuscles injected in gm	Activity of 1 gm corpuscles injected	Activity of 1 gm corpuscles secured	Hematocrit	Total corpuscle content in gm	Body weight in kgm	Corpuscle content per kgm body weight in gm
1*	10.7	30.000	62.6	63	5180	78.8	65.7
2	6.16	14.750	38.3	48.8	2370	61.8	38.3
3	6.98	14.500	36.2	49.6	2800	70.0	40.0
4	8.92	19.900	81.2	47.6	2190	61.8	35.4
5	8.59	15.600	45.7	45.8	2930	64.9	45.1
6	6.85	12.500	32.9	46.9	2600	67.0	38.8
7	8.30	15.000	64.1	42.0	1940	57.2	33.9
8	7.77	15.000	60.0	42.7	1940	57.8	33.6
9	7.49	15.000	58.9	38.2	1910	57.8	33.0
10	8.10	15.000	64.4	42.3	1890	59.1	32.0
11	7.06	15.000	55.1	37.9	1920	60.0	32.0
12	9.05	15.000	58.0	46.5	2340	69.5	33.7
13	8.96	15.000	58.9	46.5	2280	54.0	42.2
14	6.28	15.000	49.1	37.0	1920	60.0	32.0
15	7.97	15.000	57.0	44.9	2100	69.5	30.2
16	9.00	15.000	64.3	42.8	2100	54.0	38.9
17	7.18	15.000	55.5	40.5	1940	64.0	30.3
18	9.36	15.000	47.9	47.1	2930	72.0	40.7
19	8.66	15.000	62.2	44.4	2090	54.0	38.7
20	8.69	15.000	61.0	44.6	2140	69.5	30.8
21	8.88	15.000	47.5	43.3	2810	72.0	39.0
22	8.53	15.000	48.5	44.6	2640	72.0	36.7

Mean value**

* No. 1 was a patient suffering from polycythemia. The corpuscle content of this patient is not included in the average.

** Mostly lean subjects were investigated.

rhage, plus the volume of erythrocytes removed, the hematocrit reading gives the correct corpuscle—plasma ratio of the whole blood; if the corpuscle volume before haemorrhage is greater than the corpuscle volume after bleeding, plus the volume of red corpuscles removed, the hematocrit reading does not represent the corpuscle—plasma ratio of the blood of the whole body.

STEAD and EBERT (1941) who recently carried out such bleeding experiments found that, in normal human subjects, 72 hours after venesection, the red corpuscle volume always appears lower than the red corpuscle volume predicted from the pre-haemorrhage red corpuscle volume and the volume of red corpuscles removed. The changes in hematocrit reading are, however, relatively small. Later, STEAD and EBERT experimented with dogs in which a marked drop in hematocrit reading was produced by massive bleeding. The spleens were removed as, under certain conditions, the spleens of dogs discharge blood rich in corpuscles into the circulation. In experiments in which about half of the red corpuscles were removed, it was found that, while from the hematocrit reading and the plasma volume a red corpuscle volume of 1390 cm^3 was calculated, the volume of red corpuscles removed, plus the red corpuscle volume after bleeding, constituted 1039 cm^3 , only. From this result, these experimenters conclude that, when the hematocrit reading is approximately 50, the red corpuscle volume calculated from the plasma volume and the hematocrit reading is approximately 25 per cent higher than the true red corpuscle volume.

TABLE 6. — CORPUSCLE CONTENT OBTAINED WHEN USING
DIFFERENT METHODS

No.	gm corpuscle content obtained, when using		
	CO method	Dye method	^{32}P method
4	2640		2190
5	2850		2930
6	3090		2600
7	2700	2240	1940
8	2480	2290	1940
9	2850		1910
10	3250	1960	1890
11	2560	1940	1920
12	3350		2340
13	3350	2740	2280
14	2340	1990	1920
15	3020	2340	2100
16	2530		2100
17	2520	2210	1940
18	2940		2930
19	2130		2090
20	2560		2140
21	2800		2180
22	2340		2640
Mean value of those cases in which all three methods were applied:			
	2830	2320	1990

Our results, based on an entirely different method, support the conclusion drawn by WHIPPLE and his colleagues and by STEAD and EBERT. We can account for the discrepancy between the blood volume obtained from the corpuscle (^{32}P) volume and the plasma (dye) volume and the corpuscle (^{32}P) volume and the hematocrit value, respectively, by assuming that the red corpuscle content of the blood samples is about 18 per cent higher than the average corpuscle content of the circulating blood.

Blood volume

A correct figure of the blood volume is obtained if we add to the corpuscle volume supplied by the ^{32}P method, the plasma volume found with the dye method (cf. Table 7). This procedure is independent of the hematocrit figure though based on the assumption that the dye method supplies us with a correct value for the total plasma volume.

TABLE 7. — BLOOD VOLUME

No.	Corpuscle volume determined by the ^{32}P method + plasma volume determined by the dye method	Blood volume determined by the dye method	Blood volume calculated from the corpuscle volume determined by the ^{32}P method and the hematocrit figure	Blood volume calculated from the corpuscle volume determined by the CO method and the hematocrit figure
7	4930	5330	4300	5940
8	4830	5340	4240	5380
10	4420	4630	4140	7110
11	4910	5120	4690	6480
13	5110	5880	4540	6670
14	5170	5370	4760	5860
15	4810	5220	4330	6240
17	5130	5460	4430	5760
Mean value	4910	5300	4430	6180

If we determine the blood volume by the dye volume, i. e. if we calculate the blood volume from the plasma volume and the hematocrit figure, we get a value which obviously is too high. As the corpuscle content of the total circulating blood is lower than the corpuscle content of the blood sample used in obtaining the hematocrit figure, we overestimate the corpuscle volume and, consequently, also the blood volume. This may be seen from the following example. Let us assume the hematocrit figure to be 50. If we then add to the plasma volume, 50, an equal corpuscle volume, we obtain a blood volume figure = 100. In view of the fact that the corpuscle content of the circulating blood is about 18 per cent lower than the corpuscle content of the sample secured for hematocrit determination, we should add to 50 (plasma volume) 0.82×50 (corpuscle volume) and, thus, find 91 for the blood volume. Consequently,

the correct value of the blood volume makes out 91 per cent of the value determined by the dye method.

On the other hand, when the blood volume is calculated from the corpuscle volume determined by the ^{32}P method and the hematocrit figure, we obviously underestimate the blood volume. Now, if we overestimate the hematocrit value, we underestimate the share of the plasma in building up the blood and, thus, we underestimate the blood volume.

If the CO method would supply us with a correct value for the corpuscle content of the circulation, the blood volume calculated from the corpuscle volume and the hematocrit figure would be too low. However, as the CO method provides us with a too high value for the corpuscle volume, the opposite is the case. The error due to the uptake of CO by other compounds than the hemoglobin present in the circulating blood, overcompensates the error due to an underestimation of the plasma content of the blood.

Determination of the red corpuscle volume by using radio-iron as an indicator

While HAHN and HEVESY (1940) and HEVESY and ZERAHN (1942) carried out a determination of the corpuscle content of the rabbit and the hen by labelling the corpuscles with radio-phosphorus, HAHN, BALFOUR, ROSS, BALE and WHIPPLE (1941) used radio-iron as an indicator in experiments with dogs. Radio-iron is more stably bound in the corpuscles than radio-phosphorus. This fact makes possible to carry out experiments of several days duration. Experiments lasting only a few minutes lead to the same results regarding the corpuscle content as experiments lasting a few days, which proves that the mixture of the corpuscles injected with those beforehand present in the circulation had occurred already in the course of a few minutes.

In contradistinction to the labelling of corpuscles with radio-phosphorus which can be carried out in *in vitro* experiments, the labelling of corpuscles with radio-iron can only be made *in vivo*. In experiments on human subjects, it is necessary to work with donors whose blood was labelled with radio-iron. By making use of this procedure in order to obtain corpuscles of sufficient activity, very substantial radio-iron activities had to be administered. However, the preparation of even moderate iron activities — in contradistinction to that of large phosphorus activities — is a difficult task. This may be the reason why so far only animal experiments were carried out with radio-iron.

When using radio-iron as an indicator in the experiments with dogs, HAHN and his colleagues found a smaller corpuscle content than that obtained by a calculation of the corpuscle content from the plasma (dye)

volume and the hematocrit figure, the corpuscle content determined by the radio-iron method being 77 per cent of that calculated when applying the plasma (dye) and the hematocrit values. This is a result similar to that obtained by us when using the ^{32}P method.

Summary

To a blood sample taken from a human subject, a minute amount of sodium phosphate containing the radioactive phosphorus isotope ^{32}P is added. Then, the sample is shaken in a thermostat for two hours at 37°C . A part of the blood sample thus containing labelled corpuscles is reintroduced into the circulation. After the lapse of about 5 minutes, a blood sample is secured and the radioactivity of the corpuscles of this sample is compared with the radioactivity of the reintroduced corpuscles of equal weight. The ratio of the radioactivity of the two samples is a measure of the amount of corpuscles present in the circulation.

The mean value of the corpuscle content of the lean human subjects investigated was found to be 36.0 gm per kgm body weight.

In a number of cases, the plasma volume was determined by means of the dye method and the corpuscle content of the circulating blood was calculated from the plasma volume and the hematocrit figure. The figures obtained in this way, were about 18 per cent higher than those found for the corpuscle content determined according to the ^{32}P method.

When adding to the plasma volume determined with application of the dye method, the corpuscle volume found by means of the ^{32}P method, we obtain the blood volume. The value thus calculated is independent of the hematocrit figure. The values obtained by means of this direct method of determination of the blood volume are found to be about 9 per cent smaller than the values calculated from the plasma (dye) volume and the hematocrit figure.

The difference between the determined and the calculated corpuscle content (making use of the hematocrit figure) respectively between the determined and the calculated blood volume, supports the conclusion drawn by WHIPPLE and his colleagues that the hematocrit figure is no proper representative of the corpuscle content of the circulating blood, this content being smaller than the hematocrit figure. This conclusion is based on the assumption that the dye method supplies us with a correct value for the total plasma volume.

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58. APPLICATION OF ^{42}K LABELLED RED CORPUSCLES IN BLOOD VOLUME MEASUREMENTS

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LABELLING of red corpuscles by making use of ^{32}P as an indicator is made possible by the following facts.

(a) A constant fairly slow interchange of phosphate between plasma and erythrocytes takes place at body temperature.

(b) The organic labile P content of the red corpuscles is much higher than that of the plasma.

(c) The individual P atoms, and thus also the ^{32}P atoms intruded into the erythrocytes, participate in phosphorylation processes and reach rapidly an exchange equilibrium with a part of the labile P present in the red corpuscles.

Due to these facts, some of the ^{32}P atoms which when blood is shaken with labelled phosphate penetrate into the erythrocytes in the course of 1 hr, during the same time 1/10—1/20 only find their way into the plasma, after injection of the active red corpuscles into the inactive circulation.

The potassium content of the red corpuscles is also much — about 20 times — higher than that of plasma of the same weight and, correspondingly, the lifetime of an individual potassium atom in the red corpuscles is about 20 times longer than in the plasma. When introducing ^{42}K atoms into the erythrocytes, we can expect them to leave the red corpuscles at a slow rate only. The loss within 20 minutes, which amply suffice to obtain mixing between the injected and circulating blood, can be expected to be less than one per cent. This induced us to carry out determinations of the circulating blood corpuscle volume by using ^{42}K labelled red corpuscles.

EXPERIMENTAL

To 10 ml of freshly drawn heparinized blood 11—15 mgm of KCl, previously bombarded in the cyclotron and having an activity of 10—60 μC , were added and the blood was shaken for 2 hrs at 37°C. In some cases, a known aliquot of the active blood, in others red corpuscles once washed with inactive plasma were

reinjecting into the human subject under investigation. The labelled KCl was previously carefully purified from radioactive impurities, especially from ^{24}Na . Under the conditions prevailing in the Stockholm and Copenhagen cyclotrons, where the KCl applied was bombarded with deuterons, the bombardment of 1 mgm of sodium supplies about 30 times as much ^{24}Na as ^{42}K is formed by bombardment of 1 mgm potassium. (Personal communication by Mr. K. ZERAHN, to whom and to Dr. MELANDER we are much indebted for the purification of the active potassium chloride samples.) The presence of minute amounts of sodium in the KCl sample can thus become disturbing. The labelled KCl was added to blood in physiological concentration thus as 1.1% solution.

The blood samples, secured from the vena basilica at various intervals after injecting a known aliquot of the activated blood were centrifuged, the corpuscles washed with saline and their activity compared with that of a known aliquot of the blood (corpuscles) injected. In our first experiments we compared the activity of dried corpuscle and plasma samples, but, in view of the fact that ^{42}K decays with a half time of 12 hrs and the drying of the samples takes some time, we applied later Zerah's (1948) cuvettes in which the activity of fresh samples is measured. These cuvettes were also applied when determining the distribution coefficient of ^{42}K between plasma and red corpuscles *in vitro*. In these experiments to about 10 ml of freshly drawn heparinized blood kept at 37°C , 0.1 ml of a physiological sodium chloride solution containing about 0.05 mgm labelled KCl was added. After the lapse of 15 minutes shaking was interrupted and the blood centrifuged in ice-cold centrifuge tubes. Centrifugation is not to be prolonged, as corpuscles when kept at a low temperature lose some of their potassium content.

RESULTS

In Fig. 1 the activity of 1 gm red corpuscles is plotted against time after intravenous injection of labelled whole blood, while in Fig. 2 the results of experiments are seen in which washed labelled red corpuscles were injected intravenously.

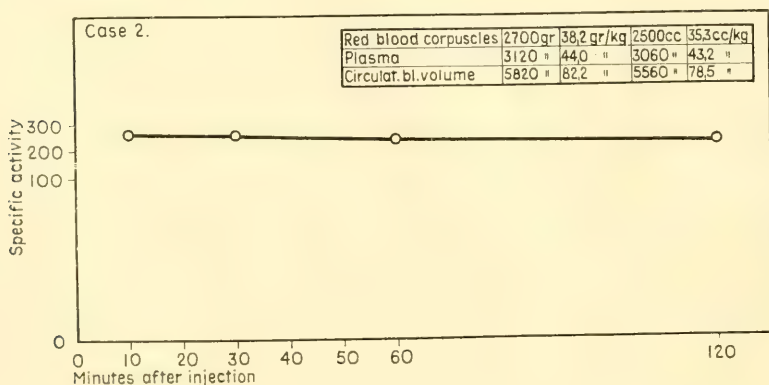


FIG. 1. Change in the ^{42}K content of red corpuscles after injection of labelled whole blood.

As distribution coefficients of ^{42}K between 1 gm fresh corpuscles and 1 gm fresh plasma at 37°C in three experiments, each taking 15 minutes, the values of 0.200, 0.209 and 0.205 were obtained. The mean value is thus 0.205. LEVI (1945) found a distribution coefficient of 1 after the lapse of 1 hr, while MULLINS and assoc. (1941) determined the time necessary to reach a 30 per cent exchange between corpuscle potassium and plasma potassium to 8.2 hr.

Since this paper went into press we became cognizant of a paper by RAKKER *et al.* (1950) and of several papers by SHEPPARD *et al.* (1950,

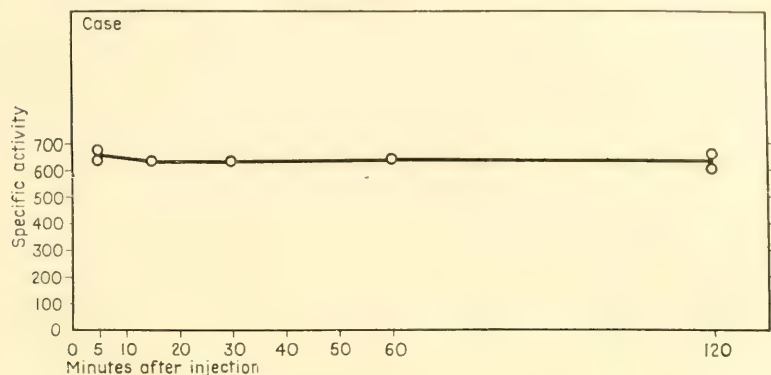


FIG. 2. Change in ^{42}K content of red corpuscles after injection of washed labelled corpuscles. Value obtained for the red blood corpuscle volume 2220 g.

1951) dealing with the *in vitro* exchange of potassium between red corpuscles and plasma. RAKKER *et al.* succeeded in maintaining erythrocytes in an essentially normal state for over 48 hours and in showing that after the lapse of that time the specific activity of plasma potassium and red corpuscle potassium becomes equal, thus a total interchange between plasma potassium and corpuscle potassium takes place within that interval. They arrive at the result that at 37°C 1.6 per cent of the potassium of the erythrocytes exchange per hour. Numerous data are stated by SHEPPARD *et al.* for the extent of potassium interchange between plasma and erythrocytes after different times and under different experimental conditions. Their data contain also figures for an incubation time of 17–18 min only. When calculating from their figures the ^{42}K loss by the red corpuscles under that time interval, we arrive to a figure of 1.0–1.3 per cent, a result which compares with the loss of 1.0 per cent observed by us in experiments taking 15 minutes. From their results obtained when incubating blood in the presence of ^{42}K for 2 min only, one can conclude that during 2 min an ^{42}K loss as large as 0.4 to 1.1 per cent takes place. On the other hand from the experiments of SHER-

PARD *et al.* taking 60 min or more, it follows that the red corpuscles lose 2 per cent only of their ^{42}K content in the course of 1 hour. These and our results indicate the presence of a small rapidly interchanging potassium fraction in the blood corpuscles. These may partly or wholly be due to a rapid interchange between the potassium of the plasma and that of those corpuscles which accumulate in the buffy coat. For canine blood where the rapidly exchanging potassium fraction is very conspicuous, SHEPPARD *et al.* succeeded in showing that at least an appreciable part of their rapidly interchanging potassium is to be looked for in the buffy coat of the isolated blood corpuscles. In experiments of very short duration the amount of ^{42}K entering from the plasma into the corpuscles during centrifugation furthermore may not be negligible. Lesion of some erythrocytes during the labelling process may also lead to some ^{42}K loss. A removal of the buffy coat which may reduce the ^{42}K loss of the erythrocytes observed shortly after their injection into the circulation, cannot be considered to be a practical proposition in routine blood volume determinations.

DISCUSSION

Different methods exist which permit to determine the rate of loss of ^{42}K by the red corpuscles. We can activate erythrocytes, and after washing them to remove the adhering plasma, shake the active red corpuscles with inactive plasma, for example for 1 hr, and subsequently determine the activity of the last mentioned plasma. The figure obtained indicates the loss of ^{42}K by the corpuscles in the course of 1 hr. This procedure has the disadvantage that washing may harm the mechanism responsible for the permeation of potassium into the erythrocytes. The permeability of potassium is known to be of a different type from that of phosphate. The strong concentration of potassium in the red corpuscles is among others easily influenced by addition of glucose to the plasma or by temperature changes. Therefore we preferred another method which is based on the following consideration.

If during a certain time a percentage of the ^{42}K of negligible weight added to the blood sample penetrated into the corpuscles, we can assume that a corresponding fraction of all potassium ions present at the start of the experiment in the plasma moved into the corpuscles as well. As the potassium concentration of the red corpuscles remains constant during the experiment (a minor deviation of this assumption would not significantly influence the result arrived at) a corresponding amount of potassium must have moved in the opposite direction, hence from the corpuscles into the plasma. We can thus determine the percentage of corpuscle potassium which moves from the corpuscles into the plasma

and also the percentage of ⁴²K which leaves the corpuscles during a given time.

From the fact that after the lapse of 15 minutes 1 gm of red corpuscles was found to contain 0.205 times as much ⁴²K as did 1 gm of plasma and 100 gm of blood to contain 40.1 gm of red corpuscles follows that, out of 100 counts added to the plasma 12 penetrated into the erythrocytes. As the potassium content of the plasma amounted to 17.8 mgm % it follows that 12% of 10.7 mgm = 1.28 mgm of those potassium atoms, which were in the plasma at the start of the experiment, are found 15 minutes later in the corpuscles, and vice versa. When making this statement we failed to consider the increase in sensitivity of the radioactive indicator in the course of the experiment. The plasma activity decreased during the experiment, took 15 minutes from 100 to 88.0, and correspondingly its mean value during the experiment was 94.0. From these figures it follows that the amount of potassium which interchanges between plasma and red corpuscles in the course of 15 minutes is not 1.28 mgm but $\frac{1.28}{0.94} = 1.36$ mgm.

As the red corpuscles contained 325 mgm % potassium, thus those present in 100 ml of blood 130 mgm the 1.36 mgm of potassium, which move from the corpuscles into the plasma in the course of 15 minutes amounts to 1.0 % of the potassium content of the erythrocytes. If we inject labelled erythrocytes into the circulation we can thus expect that 1.0% of their ⁴²K content is given off to the plasma in the course of the first 15 minutes.

The loss of ⁴²K by the active corpuscles when introduced into an inactive circulation can thus be expected to be smaller than the loss of radiophosphorus by ³²P labelled corpuscles during the same time, REEVE (1949); NYLIN (1951). This conclusion is borne out by the results of experiments in which ⁴²K labelled blood or red corpuscles were injected into the circulation and are demonstrated in Figs. 1 and 2. After injecting labelled blood into the circulation, the activity of the red corpuscles seems to remain unchanged during 1 hr within the error of the method, which amounts to about 3 %. Injection of labelled red corpuscles is followed by an initial loss of about 2 % of their ⁴²K content, followed by a further loss of 5 % in the course of the *first* 2 hours. As we found a loss of 43 % in the course of 24 hours, the mean loss of ⁴²K by the labelled erythrocytes per hour works out to be 2.1 %, a figure almost identical with that found by SHEPPARD and slightly larger only than the new figure found by RAKKER in *in vitro* experiments. That no perceptible loss of ⁴²K by the erythrocytes was observed following injection of labelled whole blood may be due to an entrance of ⁴²K from the labelled plasma into the unlabelled corpuscles, which may compensate slight losses of ⁴²K by the red corpuscles.

If we wish to apply labelled red corpuscles as a clinical tool, it does not suffice that the erythrocytes conserve their label during the determination to be carried out. It is of great importance as well that the labelling process should not take more than some minutes, that the radiation emitted by the labelled corpuscles is easily measurable and that time consuming operations as separation of the erythrocytes from plasma can be avoided. We found thorium B labelled red corpuscles to respond to all these requirements. Thorium B labelled erythrocytes can be prepared by leading a stream of oxygen containing thoron (thorium emanation) through a blood sample for a few minutes only. A large part of the thoron absorbed by the blood sample, as its half-life time amounts to 55 seconds only, decays within the sample. As a result of this disintegration radioactive ThB is formed which decays with a half time of 10.6 hr. Most of the ThB formed in the plasma is taken up by the red corpuscles. Due to this fact and also to the very speedy disappearance of ThB from the injected plasma the activity of the blood samples secured after injecting ThB labelled blood into the circulation is almost exclusively due to the activity of the red corpuscles. A separation of the red corpuscles from the plasma in the secured blood samples can thus be avoided.

Within the first 2 hr loss of ThB by the labelled corpuscles is almost negligible and for the coming hours restricted. While thorium B emits soft β -rays its daughter product thorium C with which it soon gets in exchange equilibrium emits β -rays of similar penetrability as does ^{32}P . When measuring the radioactivity of thorium B labelled erythrocytes we are thus mainly measuring the β -radiation of thorium C.

That the activity of thorium B + C decays with a half-time of 10.6 hr has the advantage that the organism is exposed to radiation for a very restricted time only after the radioactive isotope is introduced into the circulation, less than 1/5 of the isotope being present after a lapse of a day.

A more detailed description of the above outlined method and the results obtained by its application will be soon published.

Summary

Red corpuscles were labelled by adding ^{42}KCl to a blood sample kept at 37°C for 2 hr. When reinjecting the blood sample to the patient in the course of 1 hr, within the error of the blood volume determination which is 3%, no change in the activity of the red corpuscles could be observed.

Washed labelled red corpuscles injected into the circulation lost in the average 3.5% of their ^{42}K content in the course of the first hr, while the mean loss per hr in the course of 24 hr was found to be 2.1%.

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59. APPLICATION OF "THORIUM B" LABELLED RED CORPUSCLES IN BLOOD VOLUME STUDIES

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A method for determining blood volume utilizing thorium B, which has advantages over the use of ^{32}P , is described in detail. The problems of protecting the patient and operator from radiation activity are discussed in an appendix.

THE successful application of radioactive indicators in physiology caused the early introduction of a radioactive component into the erythrocytes and their use in blood volume determinations. Radio-iron labelled erythrocytes found extended application in animal experiments⁽¹⁾. These are not suitable for determination of the blood volume of humans, since iron-labelled red corpuscles can only be obtained *in vivo*, and thus the application of this method depends on the availability of iron-labelled donors.

On the other hand the use of *in vitro* with P^{32} labelled red corpuscles⁽²⁾ has found a very extended application in clinical blood volume determinations^(3, 4). This method necessitates incubation of the blood sample with radioactive phosphate for an appreciable time. It also requires centrifuging of blood samples and washing of red corpuscles, which consume some time.

An ideal radioactive method of clinical blood volume determination should fulfill the following conditions:

(a) The radioactive source should be available at a moment's notice, and should not need replacement for some years.

(b) The rays emitted by the radioactive indicator should be easily measurable.

(c) No significant loss of the radioactive indicator by the red corpuscles should take place in the circulation in the course of the experiment.

(d) The half-life of the radioactive indicator should be sufficiently long, amounting to at least several minutes, to enable a trained nurse to carry out the radioactive measurements without difficulty. The half-life should, however, be shorter, and preferably appreciably shorter than one day, since it may be necessary to repeat the blood volume determination after some time.

(e) Centrifugation of the injected or secured blood samples should not be necessary. It should suffice to compare the radioactivity of a known aliquot of the injected labelled blood, poured into a glass

cuvette, or in dry state, with that of a sample secured after injection, in order to arrive at a correct figure for the circulating blood volume of the patient.

The present communication describes a method of blood volume determination which fulfills to some extent the above conditions. It is based on an observation⁽⁵⁾ that when oxygen carrying thoron is led through a blood sample, an appreciable part of the thoron decays within the sample, and its decay product thorium B (ThB) is almost entirely taken up by the corpuscles, from which it is released at a very slow rate.*

METHOD

Radio-thorium preparations are easily available. We applied a sample prepared according to the Hahn procedure and having the activity of 2 mgm of radium. It was obtained (price £7 per millicurie) from Harwell. Such a preparation is a copious source of thoron gas (thorium emanation). It is placed in a small glass vessel of a weighing glass type. Through the stopcock of the vessel two narrow glass tubes are inserted: through one, oxygen is led into the vessel, through the other, the thoron-loaded oxygen is led (for example, for 10 minutes) into the blood sample to be activated. The narrow glass cylinder containing the blood sample is placed in a small wash bottle. Since rubber strongly absorbs thoron, rubber tubing is kept at a minimum.

Thoron absorbed by the blood sample decays with a half-time of 55 seconds and is converted into ThB and its disintegration products. Within five minutes, all thoron absorbed by the blood decays, and the activity of the blood sample is now exclusively due to the presence of ThB and its disintegration products, the activity of ThB decaying with a half-time of 10.6 hours.

While the larger part, 10 ml, for example, of the active blood sample is reinjected into the human subject, whose blood volume we wish to determine, a small fraction is applied to prepare a standard sample. In preparing such a sample, we add, for example, 0.02 gm of active blood to 2 gm of inactive blood (or saline), hemolyse the sample by adding a few grams of saponin, and pour the hemolysed blood into one of ZERAHN's cuvettes⁽⁶⁾, or preferably dry the sample and pour 100 mgm of the dry sample into an aluminium dish 1.2 cm in diameter, or, if a larger blood sample is available, preferably into a dish of larger diameter. The activity of the standard sample is then compared with the activity of a blood sample secured, for example, 10 minutes after the

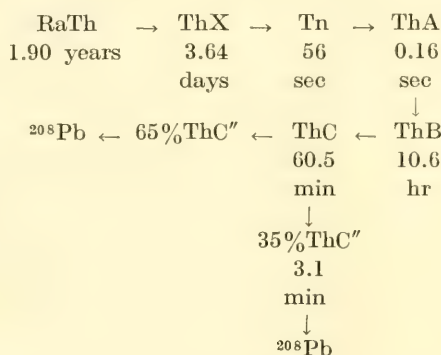
* The uptake of ThB by red cells was studied at an early date by BEIRENS and PACHUR [*Arch. exp. Path. u. Pharmacol.* **122**, 319 (1927)].

injection of the active blood took place. This sample is hemolysed as well, and treated similarly to the standard sample. It is of the greatest importance to compare the activity of dry blood samples having the same corpuscle—plasma ratio. This is facilitated by preparing the samples from hemolysed blood.

Thorium B and its Disintegration Products

Before describing the measurement of the activity of the ThB-labelled blood samples, it is appropriate to recapitulate our knowledge concerning the disintegration of the active deposit of thorium, and the radiation emitted which follows this disintegration.

The sequence of the radioactive disintegration products of radiothorium (RaTh) is shown in the following schema:



Thorium B emits, as seen from the above schema, soft β -rays, which are half-absorbed by a blood layer of 0.4 mm thickness. The disintegration products of ThB, ThC and ThC'' emit 7.5 times and 4.8 times as penetrating β -rays as ThB. Furthermore, ThC and ThC'' emit α -rays having a range of 31 μ , and ThC with a range of 56 μ , in dry blood; γ -rays are emitted as well. To what extent these radiations participate in producing ions in the Geiger tube, depends on the thickness of the layer, that of the window of the counter and the volume of the counter. The complexity of the radiation emitted by ThB and its disintegration products is in no way disturbing, as we are solely interested in the comparison of the activity of blood samples emitting the same complex type of radiation. When we use thin window counters, as applied when measuring the activity of ^{14}C , some of the strongly ionizing α -rays emitted by the dry blood sample penetrate into the counter and contribute to the total number of counts produced. When we use glass cuvettes, however, these and other soft rays are stopped by the window of the cuvette; furthermore, these, and also harder rays, are partly absorbed by the water content of fresh blood. If we wish to administer a very restricted

dose, we prefer to compare the activity of dry blood samples placed in an aluminium dish, and use thin window counters as when measuring the activity of ^{14}C .

The approximate half-value thickness of the β -rays emitted by ThB and its disintegration products in aluminium is as follows:

ThB (lead isotope)	0.10 mm
ThC (bismuth isotope)	1.2 mm
ThC" (thallium isotope)	0.77 mm

Of the disintegration products of thoron, ThA decays in a half-time of 0.16 second and can thus be disregarded. ThB, which has a half-time of 10.6 hours, takes about 6 hours to come into exchange equilibrium with its disintegration products. This is a fact of importance in interpreting the activity figures obtained for ThB-labelled blood samples.

After leading thoron through the blood for 20 minutes, for example, the maximum ThC activity of the sample is not obtained until after the lapse of 200 minutes, as shown by Table 1. In the succeeding 100 minutes, the activity decays by 3 per cent; later a decay with a half-time of 10.6 hours sets in; thus after the lapse of 21 hours, the activity is reduced to $\frac{1}{4}$, after the lapse of 42 hours to $\frac{1}{16}$ and after 63 hours to $\frac{1}{64}$. These figures indicate the maximum fraction of the injected ThB still present in the organism. The actual fraction present is, however,

TABLE 1. — CHANGE OF THE ACTIVITY OF THE BLOOD WITH TIME AFTER 20 MINUTES EXPOSURE TO A UNIFORM THORON STREAM. (ONLY THE ACTIVITY DUE TO THE RAYS EMITTED BY THORIUM C AND ITS DISINTEGRATION PRODUCTS IS CONSIDERED)

Time in minutes	Activity	Time in minutes	Activity
0	1.0000	100	6.3626
1	1.0960	120	6.7777
5	1.4682	150	7.1787
10	1.9073	200	7.4534
20	2.7060	300	7.2408
30	3.4077	400	6.6706
45	4.3009	500	6.0705
60	5.0309	600	5.4315
90	6.0998	800	4.3729

lower than the figures stated, as some ThB is lost by excretion as well as by disintegration.

Activation of Blood

We obtained the best results by the following procedure: Through 10 ml of freshly drawn heparinized blood, an oxygen stream of about 30 ml per minute percolates for 20 minutes after it has passed through

the vessel containing the radio-thorium preparation. The tube containing the blood is placed in a small wash bottle. After leaving the wash bottle, the oxygen stream passes through four more wash bottles containing vegetable oil, which absorb all or most of the thoron still present in the oxygen stream. The activated blood is then gently shaken at room temperature for 30 minutes; 9 ml are reinjected into the patient, a known aliquot of the rest being applied to prepare a standard sample as described above. We found it less advantageous to incubate blood at 37° C than at room temperature.

Instead of leading thoron containing oxygen through blood, we can dissolve the "active deposit" of thorium collected on the surface of a platinum foil in blood. Most of the ThB introduced into the blood accumulates in the corpuscles, though not to such a large extent as after thoron activation. After 30 minutes incubation at room temperature, about 6 per cent of the ThB is still found to be present in the plasma. When choosing this method of labelling red corpuscles we have, therefore, to replace the active by an inactive plasma before injecting the labelled blood. In the above experiments, the active deposit of thorium was collected* for 24 hours, on the surface of a platinum foil connected with the negative pole of a 220 volt (or preferably 300 volt) circuit. The platinum foil is then placed in the blood sample, which is gently shaken at room temperature for 10 minutes. The blood is then centrifuged, and the active plasma replaced by inactive plasma, before the blood is injected into the circulation.

Evaluation of the Activity Figures

As already mentioned, we measure, besides the counts produced by the rays emitted by ThB, to a large extent those emitted by ThC and its disintegration products. Since practically all ThB is taken up by the red corpuscles, the plasma does not contain this radioelement; it contains, however, appreciable amounts of the bismuth isotope, ThC, which does not accumulate in erythrocytes. Some of the ThC present in the plasma of the injected blood escapes in the course of the experiment through the capillary wall, while the ThC present in the standard sample has no way of escape. The ratio between the activity of the standard sample and a secured sample shortly after securing the latter does not, therefore, afford a correct measure of the circulating blood volume, as indicated by the formula stated below. We can correct for the loss of ThC from the circulation during the experiment, or we can avoid such

*The collecting vessel used is described among others in W. MAKOWER and H. GEIGER, *Practical Measurements in Radioactivity*. London (1912) and in G. HEVESY and F. A. PANETH *Manual of Radioactivity*. London (1938).

a correction by waiting for a few hours, until the ThC of the plasma, which has a half-life of one hour, has decayed. But even in this case, when comparing the activity of different samples, we have to bear in mind that a decay of half of the ThB present takes place in the course of 10.6 hours.

In evaluating the results of the activity measurements, we can avoid correcting for the change in the activity with time by comparing the activity of the sample secured with that of the standard sample, provided both change their activity with time in the same way. If the secured sample has an initial activity of 200 counts per minute, for example, we will register, in the course of 10 minutes, almost 2000 counts. In fact, as the activity has decreased in 10 minutes from 200 to 197.8 counts per minute, we will measure only 1987 counts in the course of 10 minutes or 198.7 per minute. We now measure for one minute the standard sample, having 2000 counts. The ratio between the activity of the standard sample and that of the secured sample works out then as $\frac{2000}{198.7} = 10.07$.

If greater accuracy is wanted, we again measure for 10 minutes the secured sample which has now a mean activity of 196.4, and repeat this procedure. Thus the correct activity ratio between standard and secured sample works out as

$$\frac{2000}{\left(\frac{198.7 + 196.4}{2} \right)} = \frac{2000}{197.6} = 10.12$$

As the standard sample is prepared by diluting active with inactive blood, a strongly active standard sample can be easily obtained. The measurement of the activity of such samples takes, in contrast to the

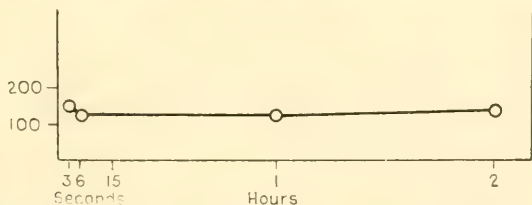


FIG. 1. Change in the activity of blood with time after injection of thorium B labelled whole blood.

secured samples, only very few minutes. When applying an automatic sample changer, which shifts five secured samples and one standard sample at 10 minute intervals, and permits reading the counts on six independent telephone counters, the following correction for decay has

to be made on the registered counts. Since counter 2 starts and finishes counting 10 minutes later than counter 1, and during this time 1.1 per cent of the ThB has decayed, the counts registered by counter 2 must be increased by 1.1 per cent of their value. Similarly the counts registered

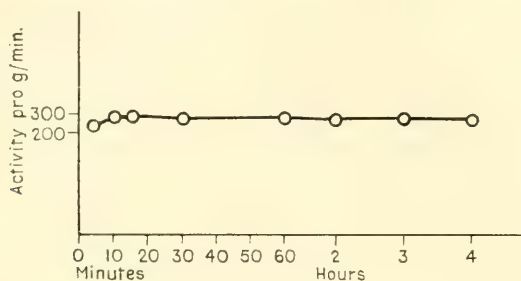


FIG. 2. Change in the activity of blood with time after injection of thorium B labelled whole blood.

by counters 3, 4, 5 and 6 have to be increased by 2.2 per cent, 3.3 per cent, 4.4 per cent and 5.5 per cent, respectively, of their value, to make them comparable with the counts registered by counter 1.

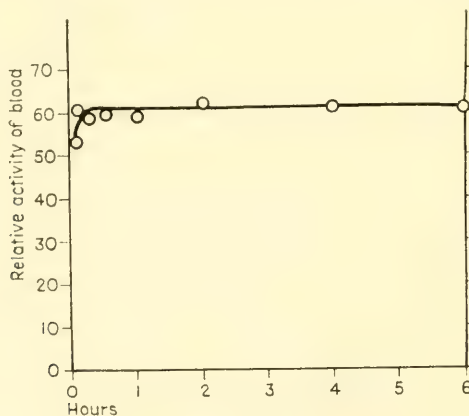


FIG. 3. Change in the activity of the blood with time in a case of polycythemia after injection of thorium B labelled whole blood

We found it very convenient to place five secured and one standard sample in an automatic sample chamber which shifts the samples every 10 minutes and registers—on six separate telephone counters—the counts produced during the night. Even blood samples of very restricted activity could be measured with satisfactory accuracy by this procedure, as seen in Table 2.

The procedure cannot be used when the result is wanted urgently. In such cases we centrifuge the blood sample before injection, and replace the active by an inactive plasma, thereby removing the disturbing ThC present in plasma. Applying this procedure we can also obtain labelled red cells by dissolving ThB collected on the surface of a platinum foil, which is then placed into the blood sample. This type of activation of the blood sample takes only a few minutes.

Decaying actinium supplies emanation (acton) as well, which in turn produces in the blood sample AcB, which has the same chemical properties as ThB, and AcC which has the same chemical properties as ThC. While one-half of the ThC present decays in one hour, the half-life of AcC is only 2.2 minutes, and this product correspondingly disappears from the plasma of activated blood in a few minutes. We are at present investigating the possibility of applying AcB-labelled red corpuscles in circulation studies.

Calculation of the Blood Volume

If we inject G gm of labelled blood, express the activity of the secured sample by P , that of the standard sample by S , and the dilution figure of the standard sample by D , the blood content of the human subject in gm X works out as $X \frac{SG}{PD}$. We then compare the activities of 100 mgm of dry blood of the secured and of the standard sample, or the activities of these samples after being poured into cuvettes. It is assumed that all ThB is concentrated in the corpuscles; in fact, about 1 to 2 per cent of the ThB content is located in the plasma. About four-fifths of this ThB leaves the circulation within a few minutes. Correspondingly, the above formula overestimates the blood content by about 1 per cent and should be replaced by

$$X = \frac{0.99 SG}{PD}$$

If, for example, $S = 2000$, $P = 200$, $G = 10$ g and $D = 0.02$, X works out as 4950 gm.

Should we wish to compare the activity of cuvettes, one of which contains hemolysed blood, while the other (the standard sample) contains a hemolysed suspension of red corpuscles in saline, owing to the somewhat lower absorbing power of the latter sample for β -rays, we have to multiply the above formula by 0.95.

While ZERAHN's cuvettes are most convenient for use in determining the circulating blood volume, the high water content of the 1.5 cm

of fresh blood placed in the cuvette absorbs much of the radiation of the sample. An activity five times as large is registered when measuring the activity of 100 mgm of dry blood as in the measurement of 1.5 ml of fresh blood placed in ZERAHN's cuvette.

EXPERIMENTAL RESULTS

Figures 1, 2 and 3 demonstrate the change in the ThB content (activity) of the circulating blood with time. The third case investigated was one of polycythemia, in which the mixing of the injected and the circulating blood was probably slower than usual, as the maximum activity of the blood was reached after only five minutes. Figures 4, 5, 6 and 7 demonstrate the results of blood volume determinations carried out by using first ThB-labelled erythrocytes, then ^{32}P -labelled red corpuscles.

When we carried out the experiments, the result of which is shown by Figures 1 to 7, we were not yet cognizant of the beneficial effect of a 30-minute incubation of the active blood previous to its injection. Nevertheless, the ThB label was found to be better preserved than the ^{32}P label. Results of the experiment in which blood incubated for 30 minutes was injected, are seen in Table 2.

TABLE 2. — CHANGE IN THE ThB CONTENT OF CIRCULATING BLOOD WITH TIME

Time between injection and sampling in minutes	Activity of dry samples secured from human subjects (Counts per minute)				Relative activities			
	1	2	3	4	1	2	3	4
3	69.3	37.2	82.4	39.7	100	100	100	100
5	72.5	57.6	82.5	39.6	104.2	101	100.1	99.7
15	72.6	36.9	84.6	39.9	104.3	98.6	102.7	100.3
30	71.8	38.1	84.9	41.3	103.4	102.4	103.2	104
60	71.4	35.7	82.1	39.1	102.9	96.2	99.7	98.4
Standard sample	775	1783	4113	204	1120	4780	4990	513

In this experiment, in the course of 57 minutes, the ThB loss by the circulating blood was found to be -3.0 per cent, $+4.3$ per cent, $+0.4$ per cent, and 1.5 per cent, respectively, which compares with a loss of about $+6$ per cent in the case of ^{32}P -labelled red corpuscles. In the course of 24 hours, one-third to one-fourth of the ThB content of the red cells is given off, which compares with a 50 per cent loss of ^{32}P by the labelled erythrocytes.⁽⁷⁾

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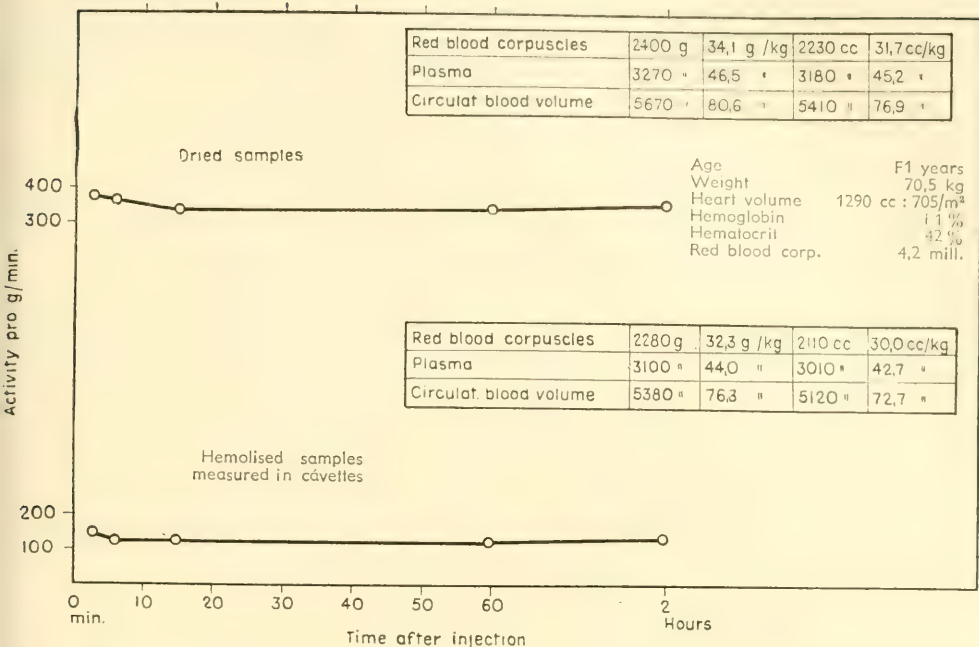


FIG. 4. Comparisons of data obtained by injections of thorium B labelled blood cells suspended in inactive plasma (above) and P³² labelled cells (below).

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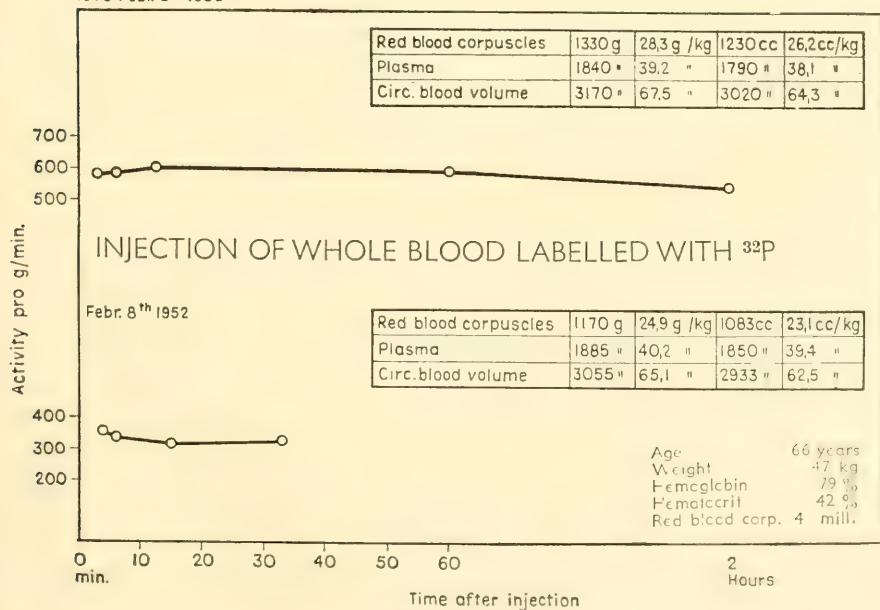


FIG. 5. Comparisons of data obtained by injections of thorium B labelled blood cells suspended in inactive plasma (above) and ³²P labelled cells (below).

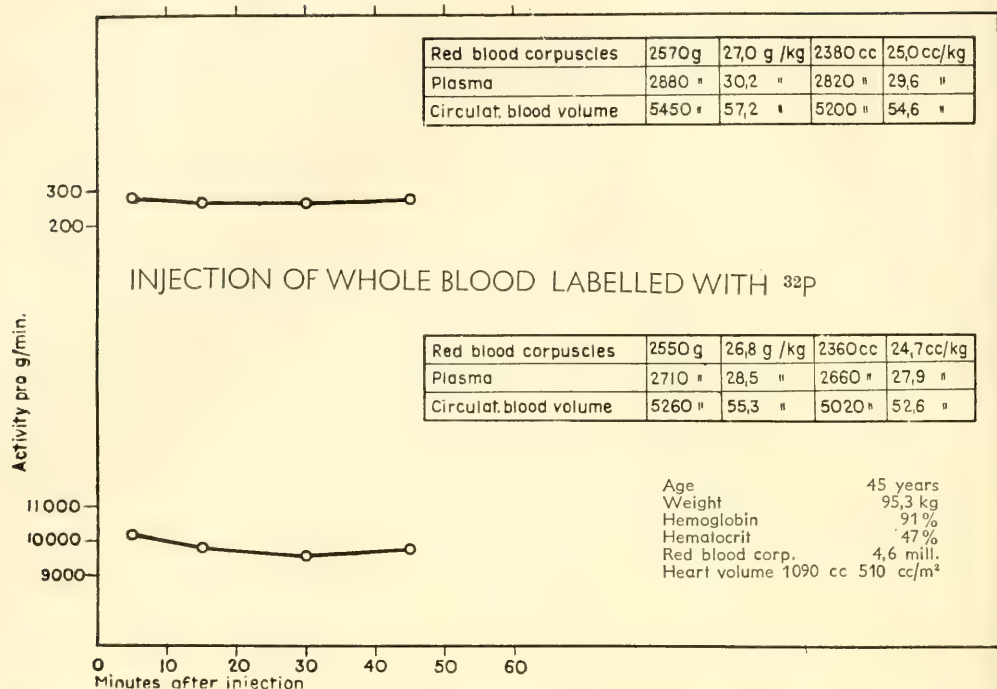
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FIG. 6. Comparisons of data obtained by injections of thorium B labelled blood cells suspended in inactive plasma (above) and ^{32}P labelled cells (below).

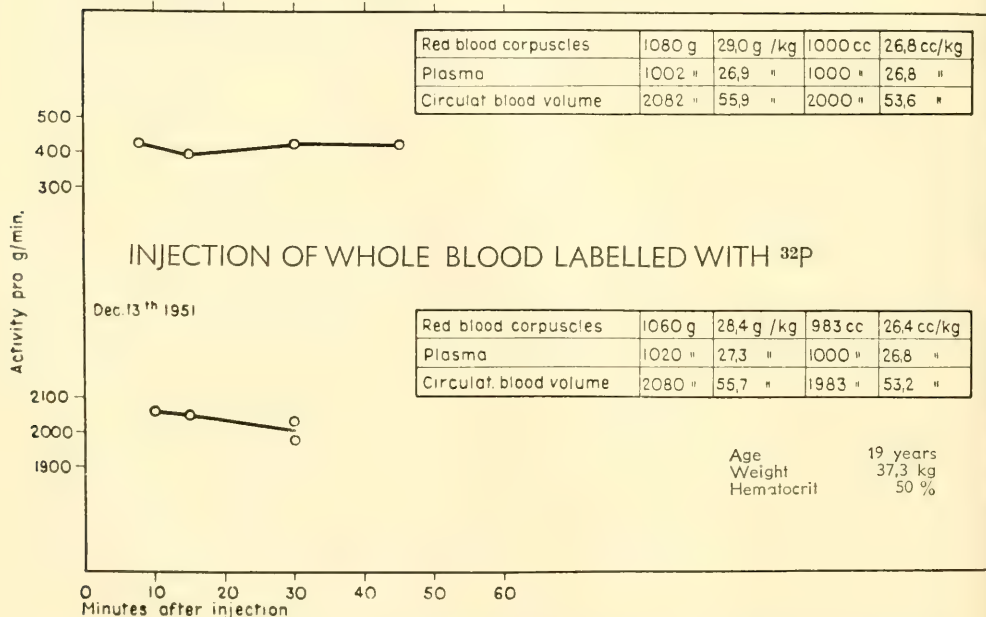
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FIG. 7. Comparisons of data obtained by injections of thorium B labelled blood cells suspended in inactive plasma (above) and ^{32}P labelled cells (below).

TABLE 3. — ThB CONTENT OF 1 gm OF FRESH PLASMA. EXPRESSED IN PERCENTAGE OF THE ThB CONTENT OF 1 gm OF FRESH CORPUSCLES, AFTER INCUBATION OF THE ACTIVE BLOOD FOR 20 MINUTES AT 37° C

Time in minutes of the passage of the thoron stream before incubation	Percentage of ThB present in plasma
15	2.4
30	1.3
45	0.97
60	0.72
75	0.63
90	0.53
105	0.50
120	0.50
10	1.6
30	0.83
10	1.2
30	0.86
30	0.76

Distribution of ThB Between Red Corpuscles and Plasma

Immediately after leading thoron-charged oxygen for 20 minutes through a blood sample, the ThB content of 1 gm of fresh plasma is found to contain 4 per cent of that of 1 gm of red corpuscles. If we

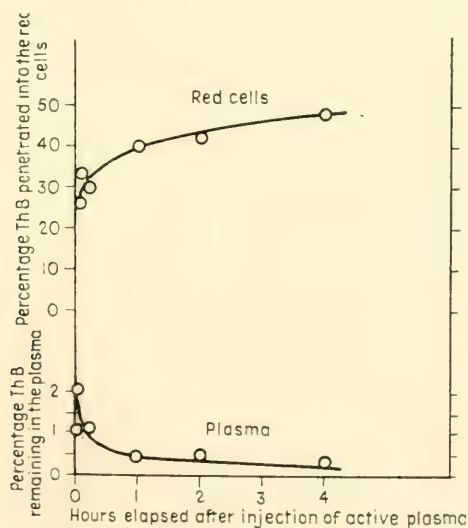


FIG. 8. Uptake of thorium B by red cells following injection of labelled plasma.

wish to avoid this restricted activity, we can replace the active plasma by inactive plasma, and inject the blood sample, the activity of which is now exclusively located in the erythrocytes. We can, however, reach almost the same result by incubating the active blood for about 20 minutes at 37° C. The percentage of ThB still present in the plasma after incubation is seen in Table 3. The ThB content of the plasma can be strongly reduced by incubating the blood for 20 minutes, as seen in Table 3.

TABLE 4. — LOSS OF ThB BY INJECTED ACTIVE PLASMA (2×10^6 COUNTS INJECTED. PLASMA AND CORPUSCLE CONTENT OF THE HUMAN SUBJECT = 3020 gm AND 1990 gm RESPECTIVELY)

Time in minutes after injection	Per cent ThB still present in the circulating plasma	Per cent ThB located in the circulating red corpuscles
5	1.25	18.5
30	0.61	18.9
60	0.30	20.8
120	0.21	20.8
24 hours	0	25.6

In incubating active corpuscles with inactive plasma *in vitro* for 1 hour, 1 to 2 per cent of the ThB of the corpuscles enter the plasma phase.

Loss of ThB by Injected Active Plasma

ThB present in the injected plasma is lost at a remarkable rate, as shown by the data of Table 4 and in Figure 8. Part of lost ThB penetrates the capillary wall, and part intrudes into the blood corpuscles.

In some of our experiments, we found the percentage incorporation of the injected plasma ThB in the circulating erythrocytes after the lapse of 3, 60 and 360 minutes, respectively, to be as high as 25, 40 and 45. These figures compare with 1, 5 and 9 for ^{32}P infusion into the red corpuscles following injection of ^{32}P -labelled plasma⁽⁵⁾.

Summary

1. Thorium B (ThB) is a disintegration product of the radioactive gas thoron. The procedure for preparation of ThB is described.

2. The advantages of ThB for blood volume determinations are that it accumulates to 99 per cent in red corpuscles, is released more slowly than radiophosphorus (loss less than 4 per cent in one hour) and has a half-time of 10.6 hours, which permits repeated determinations of blood volume. The radiation emitted by ThB is measured as easily as that of radioactive phosphorus.

3. The procedure for calculating blood volume is described in detail. In principle it consists in comparing the radioactivity of original whole blood samples with that of a sample removed from the patient after dilution. It requires no calculations of cell—plasma ratios; centrifugation is unnecessary.

APPENDIX

Radiation Protection and Radiation Exposure

Among the components of the active deposit of thorium, produced through decay of thorium emanation, is found thorium C'' which emits hard γ -rays. In view of the penetrating rays emitted by the radiothorium sample, the operator must be protected from the effect of this radiation. Placing the glass tube containing the sample in the center of a lead block 4.5 by 4.5 by 4.5 cm., reduces the intensity of the γ -rays emitted to about one-eighth, as 1.5 cm of lead cuts down the radiation by half.

A still more effective precaution is to increase the distance between the sample and the operator. While the γ -radiation of radiothorium having the activity of 1 mgm of radium produces, at 1 cm distance, a dose of 8.6 roentgen equivalent physicals (rep) per hour, at a distance of 100 cm, only 1/10,000 of that dose is produced.

When passed through the blood sample, the oxygen still contains some thoron. Though, owing to the short life-time of thoron, the activity released into the atmosphere is rather restricted, it is advisable to lead the oxygen stream which has already left the blood through an aggregate of wash bottles containing olive oil or some other vegetable oil, before releasing it into the atmosphere. While the distribution coefficient of thoron between water and air at 20° C amounts only to 0.26, the corresponding figure for olive oil and air is as high as 28.

Radiation dosage is always an important consideration when applying radioactive indicators. Owing to the short half-life of ThB, the patient is exposed to radiation for a much shorter time than in administering ^{32}P , for example. This difference is partly offset by the fact that the disintegration products of ThB emit α -rays, which are, in the mammalian tissue, more effective in producing radiation effects than the less densely ionizing β - and γ -rays. The maximum number of rep produced in the body of a human subject weighing 70 kgm by ThB and its disintegration products having the activity of 1 mgm of radium is the following:

ThC emits α -particles having an energy of 9.42×10^{-6} erg. A dose of 1 roentgen equivalent physical (rep) corresponds to the absorption of 93 ergs/gm tissue. This dose is thus produced by 0.94×10^7 α -particles; 1 microcurie emits 3.2×10^9 α -particles per day, producing 3.4×10^2 rep. Assuming the weight of the human subject to be 70 kgm, 4.8×10^{-3} rep per gram are produced.

We stated above the number of α -particles emitted by 1 microcurie in the course of a day. The effective half-life of ThB is, however, only 0.31 day. Furthermore, 35 per cent of the ThC atoms disintegrate only under emission of α -particles. The number of reps. produced by the α -particles of ThC in equilibrium with 1 microcurie of ThB thus works out to 0.52×10^{-3} rep./gm.

One microcurie of ThC., 65 per cent of which disintegrates under emission of α -particles has an energy of 1.46×10^{-5} erg, produces during its life-time 1.4×10^{-3} rep/gm.

The biologic effect of the densely ionizing α -radiation is appreciably larger than that of β - or γ -radiation producing the same number of ions. To account

for this difference, the notion of roentgen equivalent man (rem) was introduced, replacing the notion of roentgen equivalent physical (rep). For α -rays 1 rem = 20 rep (or less), while for β - and γ -rays 1 rem corresponds to 1 rep. In the course of the disintegration of 1 microcurie of ThB + ThC + ThC, thus the α -rays emitted produced not more than 4.0×10^{-2} rep. We arrive at this figure by assuming that the whole amount of ThB administered decays in the body. In fact, some ThB is excreted previous to its disintegration.

The mean energy of the β -rays emitted by ThB and its disintegration products is 0.42 Mevs. or 6.7×10^{-7} ergs per particle. By a similar calculation, as described above, we conclude that the β -particles of 1 microcurie of ThB produce during its life-time an aggregate dose of 1.1×10^{-4} rep/gm.

The upper limit of the radiation dose produced by the decay of the γ -rays of 1 microcurie of ThB and its disintegration products is obtained by assuming all γ -radiation emitted to be absorbed in the body. The mean energy of the γ -radiation emitted being 1.1 Mev., the number of rep produced per day per gm body weight works out to 3.0×10^{-4} . The upper limit of rem/gm produced by 1 microcurie of ThB + ThC + ThC. Decaying in the organism is thus $4.0 \times 10^{-2} + 1.1 \times 10^{-4} + 3.0 \times 10^{-4} = 4.4 \times 10^{-2}$. The dose actually produced lies quite appreciably below this figure. We applied in all our experiments less than 2 microcuries of ThB. Thus the total maximum dose administered was below 8.8×10^{-2} rem.

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COMMENT ON PAPERS 57—59

IN our first determination of the erythrocyte volume in 1940 we availed ourselves of the blood of a rabbit injected with ^{32}P a few days previously. A known aliquot of the blood of this rabbit was then injected into a receptor rabbit. At different times after injection, the activity of the total acid-soluble P extracted from an aliquot of the receptor blood was compared with the corresponding activity of the same aliquot of the donor blood. In some of the experiments not the activities of the acid-soluble fractions but those of the phosphatide fractions were compared. The device described (paper 51) necessitates the availability of a donor and thus could not be applied in clinical red corpuscle volume determinations. We, therefore, replaced, in 1942 the above procedure by an improved one in which the acid-soluble phosphorus compounds present in the erythrocytes are tagged in the course of incubation of blood at body temperature for 1—2 hr, and a known volume of this blood containing tagged erythrocytes is then reinjected into the circulation (paper 58). Within the first 10 min after injection, which under physiological conditions amply suffices to carry out a determination, of the red corpuscle volume, the loss of ^{32}P by the injected human red corpuscles is insignificant; after the lapse of 1 hr the loss is about 6 per cent. We injected in our studies labelled whole blood to compensate the small ^{32}P by a corresponding incorporation of plasma ^{32}P into the circulating erythrocytes (paper 52). Later, mostly labelled red corpuscles suspended in inactive plasma or saline were injected in such determinations. Comparing the results obtained when injecting whole blood or suspended erythrocytes, no significant difference was found by HANS BÖHR (1954). ^{32}P *in vitro* tagged erythrocytes found a very extended application in the forties and the early fifties. At present, radiochromate labelled erythrocytes are mostly applied in the determination of red corpuscle volume. The most extensive clinical application of ^{32}P tagged red corpuscles starting at a very early date is due to NYLIN. ^{32}P labelled red corpuscles found also application in his recent studies on brain circulation.

In vivo labelling of erythrocytes through incorporation of radio-iron into their haemoglobin was introduced by HAHN *et al.* (1941), thus soon after the first application of *in vivo* ^{32}P labelled erythrocytes in blood volume determinations (paper 51). Radio-iron *in vivo* tagged erythrocytes proved to be very useful in animal experiments, but were not suitable for clinical application (since they necessitated a ^{59}Fe activated donor).

Also ^{42}K labelled erythrocytes found application in red corpuscle determinations (paper 58). ^{42}K labelling of red corpuscles *in vitro* takes place about as rapidly as that of ^{32}P labelling. ^{42}K is retained better, and as its life-time is about thirty times shorter than that of ^{32}P , a human subject is exposed, after injection of ^{42}K labelled erythrocytes, to a smaller radiation dose than after injection of ^{32}P labelled red corpuscles of the same activity. The disadvantage of the method is that fresh ^{42}K must be procured about every second day. An isotope with a still shorter life-time than ^{42}K is the lead isotope ThB. By letting an oxygen stream strike a radio-thorium sample, the former carries gaseous thoron given off by radio-thorium. The thoron having a half-life of 56 sec only decays to an appreciable extent when passing a blood stream producing ThB, which is almost quantitatively taken up by the erythrocytes (paper 53 and ALEXANDER, 1953). Activation

of the erythrocytes takes only minutes, and the ThB is better retained by the red corpuscles than ^{32}P or ^{42}K . Since the plasma is practically inactive, it does not have to be removed before injection. This very convenient method (59) has two drawbacks. Emanating radio-thorium preparations which are precipitated with iron hydroxide lose with time much of their emanating power, as already noticed by OTTO HAHN to whom this method of preparations is due. The disintegration products of ThB, which are the ThC products, emit α -particles. These densely ionizing particles are several times as biologically effective than β - or γ -rays. The increasing biological activity of the α -rays is compensated by the short life-time of ThB, and correspondingly the subject investigated is exposed to radiation for a short time only. The not entirely unjustified reluctance to administer α -rays emitting radioactive substances to human subjects, is responsible for the fact that this method has not found an extensive application.

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60. CANCER ANAEMIA

Paper read at the Lindau Conference of Nobel Laureates in 1957

NUMEROUS data are to be found in the literature on the haemoglobin concentration in the blood of cancer patients; for example, SHEN and HOMBURGER,⁽¹⁾ found that more than 60 per cent of the cancer patients whom they studied showed a haemoglobin concentration in the blood which was 20 per cent or more below the normal.

In the study of animals with cancer it was also found, even shortly after the inoculation of rats, mice, rabbits and other animals with cancer cells, that the haemoglobin concentration in the blood decreases. Five days after the inoculation of rats with Ehrlich's mouse carcinoma the number of red blood corpuscles fell from 8.55×10^6 to 7.62×10^6 per mm³ and the haemoglobin concentration in the blood from 96 per cent to 74 per cent. When 13 days had elapsed the haemoglobin concentration was only 46 per cent⁽²⁾. Seven days after the inoculation of mice with mammary-gland adenocarcinoma, a tumour was developed which had a diameter of 0.5 cm and the haemoglobin concentration had decreased by about 7 per cent; when the tumour diameter had reached 3 cm the haemoglobin concentration had fallen to half the normal value.⁽³⁾

In so far as the blood volume is normal, a comparison of the haemoglobin concentrations and contents in cancer patients and healthy people yields the same result. The blood volume (plasma volume) of the cancerous organism, however, is frequently not normal but enhanced. The enhanced plasma volume is significant according to experimental data which have been obtained in our laboratory (Fig. 1). They show, among other results, the haemoglobin concentration, blood volume and the haemoglobin content, calculated from these values, for forty-two normal and cancerous mice, as determined 15 days after injection with Ehrlich Ascites cells. As a result of the 31 per cent enhanced plasma volume of the cancerous animals their blood volume is increased, and when this increase is taken into consideration it is found that the total haemoglobin content of the blood in the controls and in the cancerous animals shows no significant difference, whereas the haemoglobin concentration in the blood of the cancerous animals is decreased by 18.9

per cent. A similar result was an increased plasma volume in cancerous animals first observed by FURTH and SOBEL⁽⁴⁾ and later by EHRENSTEIN, whose studies we shall discuss later. An increased plasma volume is especially evident with tumours producing oestrogens.⁽⁵⁾

An increased plasma volume was also determined in numerous patients suffering from cancer. BERLIN and co-workers⁽⁶⁾ studied 66 cases and found that more than one-quarter of them had a plasma volume greater than

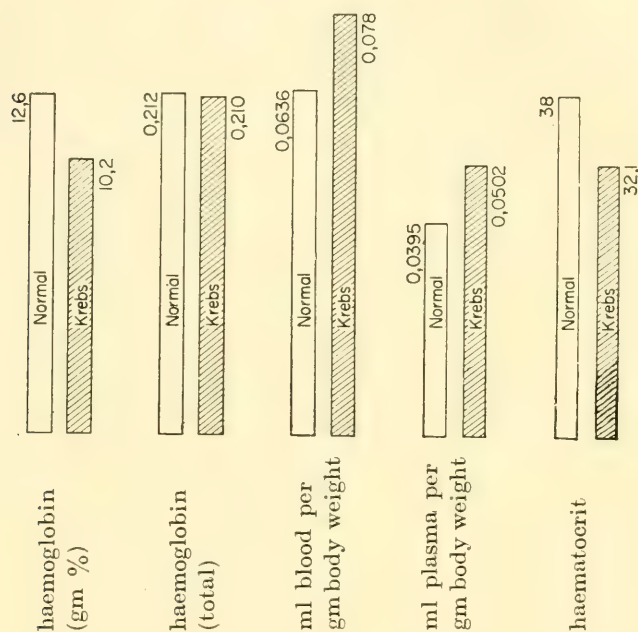


FIG. 1. Haemoglobin concentration and total haemoglobin content in the blood of the mouse. Normal — normal. Krebs — cancerous.

46 ml per kgm. They considered plasma volumes lying between 32 and 46 ml per kgm body weight to be normal. KELLY and his collaborators⁽⁷⁾ observed a plasma volume of 63 ml per kgm in thirty-three patients with advanced cancer. The total water volume of these patients was normal.

A decrease in concentration of plasma proteins usually goes hand in hand with an increase in plasma volume. Fig. 2 shows the concentration of plasma protein in normal and cancerous mice as determined by LOCKNER in our laboratory. BERNFELD and HOMBURGER⁽⁸⁾ found a 24.5 per cent decrease in albumen concentration in the plasma of mice with tumours. The increase in plasma volume is essentially due to entry of water and salts into the vascular volume.

A reduced haemoglobin concentration in the blood of cancerpatients can, however, often be observed, which is not attributable only to an increase in the plasma volume. ROSS and his co-workers⁽¹⁰⁾, for instance,

have been able to prove anaemia in three-quarters of the leukaemia or advanced cancer cases which they have studied, even when taking into account the blood volume. Since bleeding was non-existent in these patients there was no visible haemolytic anaemia or anaemia due to defective nourishment, and the anaemia was clearly attributable to curtailment of the life-time of the red blood corpuscles. It has repeatedly been demonstrated that the life-time of red blood corpuscles is often curtailed in cancer and leukaemia. BERLIN and his co-workers⁽¹¹⁾ observed

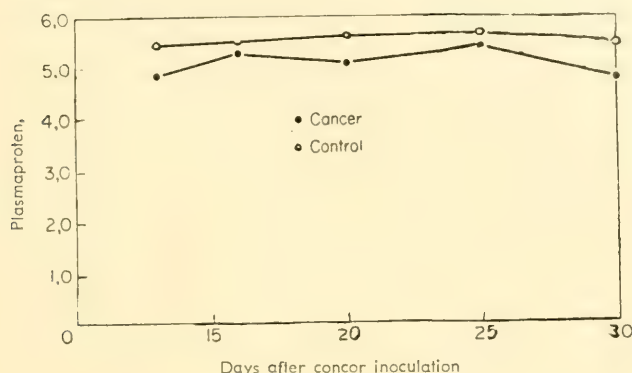


FIG. 2. Concentration of plasma protein in normal and cancerous mice.

a life-time of only 18 days for the red blood corpuscles in a patient suffering from leukaemia instead of the 120 days in a healthy subject. In these studies the erythrocytes were labelled with ^{14}C . Glycine labelled with ^{14}C was given to the patient. For some days there was a decided incorporation of ^{14}C into the haemoglobin, but this later decreased to a very low value. In these investigations the erythrocytes formed in the course of the early days, were mainly labelled with ^{14}C . This is the best method of labelling the red blood corpuscles, but it is only rarely applied in clinical studies because one is unwilling to introduce long-lived radioactive substances into the human organism. It is true that, after the addition of $100\ \mu\text{c}$ of glycocoll-2- ^{14}C , which is sufficient for a determination of the life-time, the body is only exposed to a radiation dose of 37 mrep during the first 3 months and later to a steadily decreasing radiation⁽¹²⁾. The incorporation of ^{14}C into skeleton is very small. In experiments with mice, in our laboratory, ZACHARIAS found that only $1/20,000$ of the injected glycine-2- ^{14}C was present in the skeleton after 6 months had elapsed. The radiation dose given to the patient is therefore quite small. A far more serious clinical disadvantage of the method, however, is that blood samples must be taken from the patient through a period of several

months because variations from normal behaviour are often observed only after such a long time. It is different when red blood corpuscles are labelled with radioactive chromate⁽¹³⁾. The labelling is far less stable than in the case of ^{14}C . One-half of the chromium leaves the healthy red blood corpuscles in a time of from 26 to 32 days. The labelling chromium disappears even earlier from the red blood corpuscles when the life-time of the erythrocytes is curtailed. This method is of widespread application in the study of the life-time of erythrocytes in cancer patients. It is sufficient, in this instance, to observe the patient for a few weeks. ASHBY's serological method has also been applied repeatedly to determining the life-time. In this method, the fate of normal blood corpuscles of the 0-group, transfused from another organism, is traced in the circulatory system of the patient. Tracing the life-time of heterologous blood corpuscles in the circulatory system of the acceptor, however, may lead to erroneous results in certain conditions.

Curtailment of the life-time of erythrocytes in cancer patients has often been observed with the aid of the two methods just mentioned^(14, 15, 16, 19, 10). For example, the erythrocytes in a patient with severe cancer, labelled with ^{51}Cr and investigated by KEIDERLING⁽¹⁷⁾, had already lost half of the labelled atoms after 12 days. The studies mentioned above were all performed on severe cases. We had the opportunity to study patients with cancer of the cervix, in the Gynaecological Division of the Radiumhemmet in Stockholm, which is directed by Dr. KORTMEIER; the majority of these were exposed successfully to therapy after the study and later reached a stationary condition. A study by DAL SANTO⁽¹⁸⁾ indicated that in these instances there was a curtailment of the life-time in about one-half of the cases studied.

The question now arising is how the frequently observed curtailment of the life-time of the red blood corpuscles comes about in the cancerous organism.

The red corpuscles may be exposed to extracorporeal effects in the cancerous organism and abnormal erythrocytes may also be developed in the cancerous organism. Red corpuscles have repeatedly been transferred from cancerous to normal subjects and these have not infrequently indicated a prolongation of the life-time^(16, 27, 28). The transfer of normal erythrocytes into the circulatory system of cancer patients not infrequently resulted in curtailment of the life-time of the erythrocytes. ROSS and MILLER⁽¹⁹⁾ mention that when normal blood corpuscles had circulated in the system of a neoplastic organism for 7 days and then been re-introduced into the normal organism they no longer exhibited the behaviour of normal blood corpuscles. From these results the conclusion can be made that blood corpuscles are subject to an injurious extracorporeal effect in the circulatory system of patients suffering from neoplasms.

The question of whether the curtailment of the life-time of erythrocytes is attributable to an extracorporeal effect or to their abnormal synthesis can easily be decided by the study of animals. The erythrocytes existing in the healthy organism can be labelled with ^{14}C and after incorporation, which proceeds for only a few days at a noticeable rate, the organism is inoculated with cancer cells. If the inoculation with cancer cells affects the life-time of the labelled erythrocytes, it is then certain that extracorporeal agents are playing a part. EHRENSTEIN

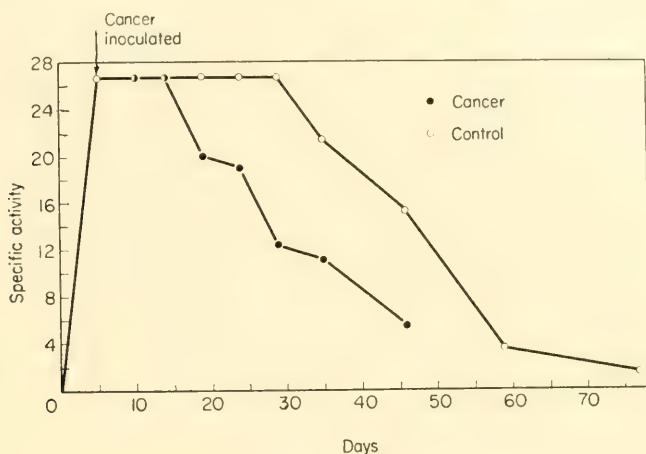


FIG. 3. Loss of ^{14}C by the haemin of red corpuscles of control mice and such inoculated with ascites cancer cells 5 days following injection of glycine- ^{14}C .

in our laboratory has injected one hundred mice, each with $1\ \mu\text{c}$ of glycocoll-2- ^{14}C , killed groups of ten mice at different times, isolated 20 mgm of haemin from the red corpuscles and compared the radioactivities of the haemin samples obtained at various times. With another batch of one hundred mice the procedure was the same, except that these were injected with Ehrlich carcinoma cells 5 days after the beginning of the experiment. As shown in Fig. 3, the life-time of the erythrocytes in the tumorous mice (tumour weight 1–3 gm) was lowered to about one-half of the normal value. Because of the rapid disappearance of haemoglobin from the circulatory system of the cancerous animal, it would be expected that they would show a lowered haemoglobin content. This was not the case; the cancerous animals had the same total of 360 mgm haemoglobin content as the controls and there was a marked decrease in the haemoglobin content just before the death of the animals. This result can be explained only by a compensation of the more rapid decay of the blood corpuscles in the neoplastic organism by a more rapid formation of erythrocytes. Figure 4, which represents the results

of other experiments by EHRENSTEIN, in which the red corpuscles were labelled in animals with tumours but not in healthy animals, shows that this holds good. The incorporation of the ^{14}C into the erythrocytes is increased fourfold in this case. The mice are able to compensate the curtailment of the life-time of red corpuscles in a quantitative manner by means of increased bone-marrow and spleen function. The cancer patient also usually shows a similar compensation. Mostly, of course, this is an incomplete compensation. We shall return to this question later.

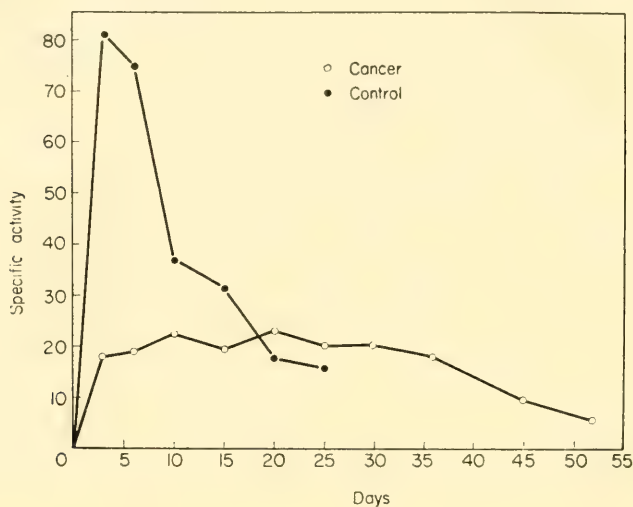


FIG. 4. Life-time of red corpuscles labelled in a mouse with carcinoma and in a normal mouse.

Figure 4 also shows that a portion of the erythrocytes formed in the animal having carcinoma decays rapidly, from which we may conclude that, apart from the extracorporeal action to which the red corpuscles are exposed in the tumourous animal, abnormal erythrocytes are also formed to some extent in such an animal.

The extracorporeal damage to the erythrocytes must clearly be attributed primarily to a plasma factor or to activation of the reticulo-endothelial system. Haemolysing substances have often been observed to exist in tumours. It has also been found⁽²⁴⁾ that the volume of the individual erythrocyte undergoes an increase due to the action of the plasma of a cancer patient; a volume increase initiates haemolysis. A range of enzymes are present in enhanced concentration in the plasma of a cancerous organism. WARBURG and CHRISTIAN^(21, 22, 23) and others observed a marked enhancement of the aldolase content in plasma when large tumours existed. One in four of the cancer patients studied, was

also observed to have an increased aldolase content in the plasma⁽²⁴⁾. Phosphatases⁽²⁵⁾ and lactic acid dehydrogenase⁽²⁶⁾ have been found, among others, at enhanced concentrations in cancerous plasma. Factors present in cancerous plasma may not exclusively be responsible for the curtailment of the life-time of red corpuscles. The R. E. system, which presumably plays only a more or less passive role in the uptake of erythrocytes which have reached the physiological end of their existence, can be activated in the neoplastic organism and can act upon the

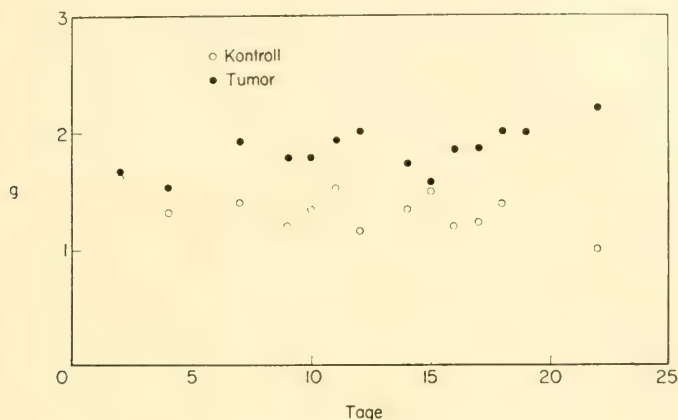


FIG. 5. Liver weights of control and tumour-bearing mice.
Kontroll — control; Tumor — tumour, Tage — days.

red corpuscles. In healthy human beings red corpuscles have an approximately constant life-time of 100—120 days and when this has been attained they are taken up by the R. E. system. P. MIESCHER⁽²⁸⁾ has sought for ^{51}Cr in the organs of rabbits into which ^{51}Cr -labelled red corpuscles had been transfused 25—35 days previously. He found the greatest concentration of ^{51}Cr in the bone marrow, with the liver and spleen next in order. It might be argued against these results that the ^{51}Cr is not firmly bound in the red corpuscles, that it escapes from the intact erythrocytes, and therefore an enrichment of ^{51}Cr in an organ can be attributed to causes other than the decay of red corpuscles in the organ concerned. Experiments performed in our laboratory by EHRENSTEIN and LOCKNER⁽²⁹⁾, with transfused erythrocytes which had been labelled with ^{59}Fe , showed a very similar result. Haemoglobin, so long as it is embedded in the intact erythrocyte, is among the most stable compounds present in the animal body and ^{59}Fe can escape from it only after damage to the erythrocyte. In the experiments mentioned above, the plasma of the acceptor-sister animal was inactive and ^{59}Fe was present only in the transfusing erythrocytes. The ^{59}Fe which they were able to detect

in the deposited iron (in ferritin and haemosiderin of the bone marrow), for example, 10 hr after transfusion, could only be derived from the erythrocytes degraded in this organ. Erythrocytes do not set free any ^{59}Fe as long as they are intact. This could be proved by performing experiments in which the labelled red corpuscles were first circulated

TABLE 1. — DISTRIBUTION OF THE ^{59}Fe FROM THE TRANSFUSED RED CORPUSCLES, WHICH WERE NOT HAEMOLYSED IN THE ACCEPTOR RABBIT, BETWEEN THE FERRITIN PLUS FERROSIDERIN FRACTIONS OF THE ORGANS

(3 hr experiment)

Organ	Percentage distribution of ^{59}Fe from the transfused red corpuscles in the ferritin plus ferrosiderin fractions of spleen, liver and bone marrow
Spleen	8
Liver	35
Bone marrow ...	57

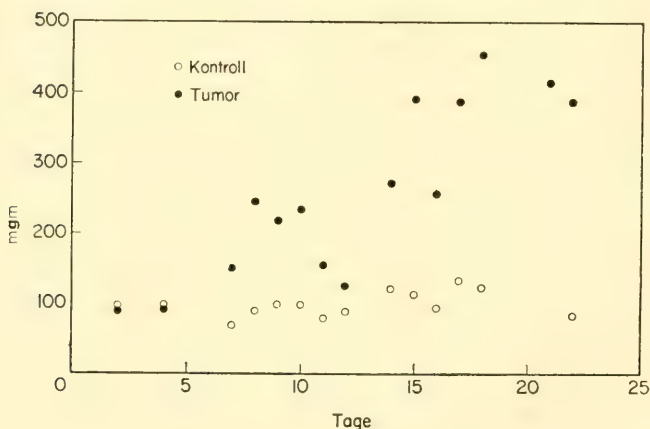


FIG. 6. Spleen weights of control and tumour-bearing mice. Kontroll — control; Tumor — tumour; — Tage — days.

for 2 days in a sister acceptor, in order to allow the escape of any possible mobile ^{59}Fe , and then adding these corpuscles to a third rabbit. These experiments yielded a result similar to that first discussed. The organ in which most of the erythrocytes end their physiological life is the bone marrow, with the liver occupying second place. One gm of spleen contained more Fe^{59} than 1 gm of any other organ. Since the weight of the spleen is very small, especially in the rabbit, the whole spleen contained much less ^{59}Fe than either the whole of the bone marrow or the whole liver (cf. Table 1).

In haemolytic anaemia and in a series of other ailments there is a quite different behaviour. In these illnesses the blood corpuscles do not attain nearly the same life-time but decay in accordance with a statistical law like that for radioactive atoms. The R. E. S. plays an active part in the death of these erythrocytes, and MISCHER⁽³¹⁾ has produced a series of arguments to support this. The cancerous mice involved in EHRENSTEIN's previously discussed experiments had greatly enlarged spleen and liver, with average weight increases for these organs amounting to 153 and 42 per cent (Figs. 5 and 6). A study of mice having spontaneous tumour yielded a similar result. Furthermore, the incorporation of ^{32}P into the desoxyribonucleic acid of the liver and spleen was found to be markedly increased in adult mice inoculated with breast cancer (KELLY and JONES^(32, 33)). Such incorporation into the liver and to some extent also to the spleen, must be attributed essentially to additional cell formation.

An investigation by BERLIN, LAWRENCE and ELMINGER⁽³⁵⁾ shows, among other data, that the erythrocytes in a diseased enlargement of the spleen frequently decay roughly in accordance with a statistical law, but that as soon as the spleen is removed all the red corpuscles attain approximately the same life-time. On the other hand, removal of the spleen from a healthy person, which has been done repeatedly after accidents causing a rupture of the spleen, does not noticeably affect the life-time of the erythrocytes⁽³⁶⁾. These results demonstrate quite clearly the great difference in the parts played by the spleen in physiological and pathological decay of the red corpuscles.

METABOLISM OF IRON IN THE CANCEROUS ORGANISM

The study of the metabolism of iron in the cancerous organism can lead up to valuable information concerning the curtailment of the life-time, the existence of hyperplasia of the bone marrow, and so on. In contrast to the determination of the life-time of red corpuscles, which has been discussed in the previous pages, such investigations require only a few hours, a fact to which great importance attaches in clinical studies. The iron content of the plasma is frequently decreased to a smaller extent than the iron concentration in the plasma, since indeed the plasma volume is sometimes increased (see p. 598). Even when this increase is taken into account, there is often a lower content of iron in the plasma of cancer patients. The magnitude of the iron content of the plasma is determined essentially by the amounts of iron discharging, chiefly to the bone marrow, and flowing in from the iron deposit. If the rate of formation of red corpuscles is accelerated, as it is often the case with cancer

patients, the iron metabolism in the plasma will be under greater strain. A lower level of plasma iron will be established if more iron is discharged from the plasma than is yielded by the supplying organs. The organism, however, is capable of compensating, within a wide range, for the lower level of iron by an accelerated output of iron. This is shown in Fig. 7, which is taken from work performed by DAL SANTO in our laboratory in which he studied women, suffering from cancer of the cervix, who were patients of the Kottmeier Clinic at the Radiumhemmet, Stockholm;

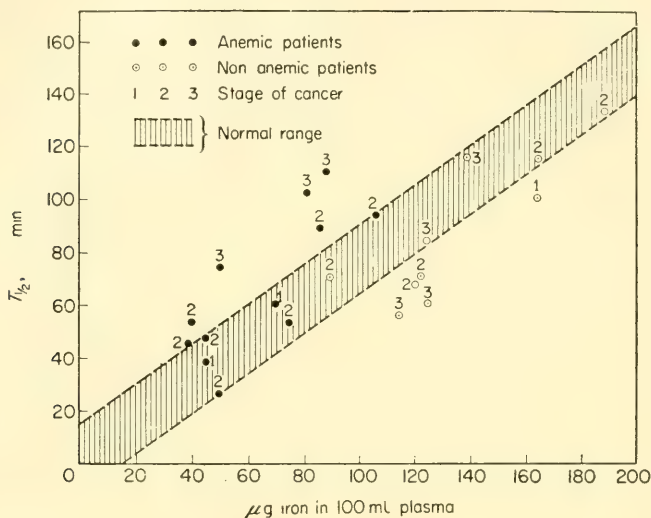


FIG. 7. The decline of plasma iron, labelled with ^{59}Fe , as a function of the iron concentration in the plasma.

the method applied was that due to HUFF and co-workers⁽³⁸⁾. In several cases the iron moves too rapidly and the $T_{1/2}$ values turn out too low. When more prolific bleeding can be excluded, such a result is a very plausible indirect proof of the curtailment of the life-time of erythrocytes. In order to compensate for a curtailment of the life-time of the erythrocytes by about one-fourth of their normal value, it would be necessary to add the same extra amount of iron as that which would compensate a chronic bleeding of about 10 ml/day.

In a series of cases (Fig. 7), the iron moves too slowly and the $T_{1/2}$ -values are too high. All of these patients were anaemic. ROSS and MILLER⁽¹⁹⁾ in a study of twenty-eight severe cases, found twelve with a supernormal and only three with a subnormal iron metabolism. In nearly half of the cases investigated there was a definitely detectable curtailment of the erythrocyte life-time.

METABOLISM OF IRON BEFORE AND AFTER TREATMENT WITH RADIATION

Fourteen of the cervical cancer patients, studied by DAL SANTO, who had been found clinically healed after single radiation treatments by the chief of the clinic KOTTMEIER, were investigated by LOCKNER about 1 year later. The high iron metabolism and also the increased plasma volume were fully or partly normalized. The iron transport was found lowered from 0.96 mgm/hr per litre R. B. C. to 0.76 mgm ($P < 0.001$); the plasma volume and the volume calculated per kgm of body weight were also lower. A minor fraction of the enhanced iron metabolism observed before the treatment can possibly be attributed to bleeding. Most of the enhancement of the iron metabolism, however, cannot be explained by bleeding anaemia; rather must we regard the curtailment of the life-time of the erythrocytes, as is also shown by life determinations using red corpuscles labelled with ^{51}Cr , to some extent, to be responsible. This curtailment producing anoxia leads to an increased iron turnover.

Summary

The chief cause of cancer anaemia is a curtailed life-time of the red corpuscles as is shown by various researches performed in this field. The curtailed life-time is partly the result of extracorporeal factors, such as the presence of destructive agencies in the plasma and activation of the reticulo-endothelial system, and partly to a formation of abnormal blood corpuscles. The curtailed life-time of the blood corpuscles is partly or wholly compensated by a more rapid re-formation of erythrocytes.

The erythrocytes of many of the cervical cancer patients who were studied had a curtailed life-time before treatment; the iron metabolism was shown to be increased as a result of the presence of hyperplasia of the bone marrow; the plasma volume also was enhanced. A year after radiation treatment there was mostly a normal life-time of erythrocytes in the now clinically healthy people. The iron metabolism and the plasma volume exhibited normal or reduced values.

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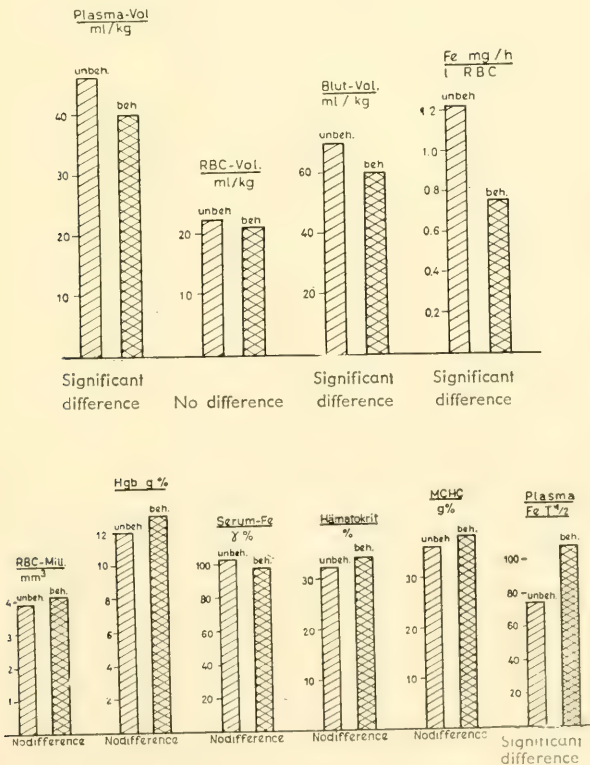
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COMMENT ON PAPER 60

In the course of the last two years, the plasma iron turnover of 27 women suffering from cancer of the uterine cervix was determined in our laboratory prior to and about one year after successful radiotherapeutic treatment by Dr. Kottmeier, director of the gynaecological department of Radiumhemmet. With the exception of 2 patients all have shown a significantly decreased iron turnover after treatment. The mean value of the iron turnover being prior to treatment 1.22 and after successful treatment 0.75 mgm Fe(hr)/RBC ($p < 0.001$). The mean haemopoietic data of all treated patients are seen from the figures stated below. The first column indicates data prior to, the second column such after treatment. The patients were slightly anaemic only and their plasma iron concentration was normal, both before and after treatment. Iron turnover figures and also plasma volume differ significantly prior to and after therapy. All recidivous patients investigated were showing turnover values between 1.07 and 1.32 mgm Fe(hr)/RBC.

A detailed presentation of the results obtained is given by Lockner.

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61. EFFECT OF ADRENALINE ON THE INTERACTION BETWEEN PLASMA AND TISSUE CONSTITUENTS

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A LARGE number of mice were injected intraperitoneally with labelled phosphate, partly after previous administration of adrenaline, partly without, and 15 min later the distribution of ^{32}P between liver, muscles, skeleton, and blood plasma was determined. The ^{32}P content of the plasma was found to be markedly depressed in the adrenaline injected mice. Besides the rate of extrusion of the labelled phosphate from the circulating plasma, its rate of resorption into the circulation may be influenced as well. We injected a tracer amount of labelled phosphate into the circulation of the rabbit with the aim of eliminating the last mentioned effect and followed its rate of disappearance. Adrenaline was found to increase very markedly the rate of extrusion of ^{32}P from the vascular bed also in this case.

The rate at which phosphate passes the capillary wall is a very high one. In order to investigate the effect of adrenaline on a slowly penetrating compound we studied the rate of extrusion of labelled iron injected intravenously as iron β -globulin in the controls and in the adrenaline injected rabbits. Administration of adrenaline was found to accelerate the exodus of the slowly disappearing ^{59}Fe from the circulation. We determined furthermore the rate of exodus of radiosodium from the circulation.

EXPERIMENTAL

In each of our first thirteen experiments, 10 controls and 10 adrenaline administered mice of about 18 gram were injected with 0.1 ml saline containing 0.3 microcurie of ^{32}P present as carrier-free sodium phosphate. 15 min later, the mice were decapitated and the ^{32}P content of dry plasma, liver, femur, and gastrocnemius tissue of the same weight of controls and adrenaline administered mice was compared. 2 to 20 microgram of adrenaline chloride dissolved in 0.1 ml saline were injected subcutaneously to every second mouse 10–40 min before administration of ^{32}P . The controls were injected at a corresponding time with 0.1 ml saline which did not contain adrenaline. In the later series of these experiments, 10 microgram of adrenaline dissolved in 0.1 ml of saline were injected

subcutaneously to every second mouse 20 min before the administration of ^{32}P . After the lapse of a further 15 min the mice were decapitated. The adrenaline preparation (exadrin) used in our experiments was a generous gift of Astra.

In the experiment in which the rate of disappearance of the intravenously injected ^{32}P was followed, rabbits weighing 2–3 kgm and previously injected with urethane (1.5 gm per kgm body weight) were injected into the vena jugularis with 0.1 ml of saline containing 0.1 mgm of ^{31}P and ^{32}P of 25 μC activity. Plasma samples of about 0.5 ml were secured at intervals from the carotid. The technique used was thus the same as applied by HAHN and one of us (1941), by FLEXNER and his colleagues (1942), and numerous other experimentors. Another group of sister rabbits was injected subcutaneously 24 min. before the start of the experiment with 40 microgram of adrenaline.

In our investigations on the effect of adrenaline on ^{59}Fe extrusion, we injected labelled FeCl_3 containing about 3 microgram of iron and 1 microcurie of activity into the ear vein of a rabbit and, 1¼ hour later, when its plasma contained the ^{59}Fe almost exclusively as β -globulin, 6 ml of the plasma of this rabbit were transfused to a sister rabbit. Pharmacological doses of adrenaline were administered by subcutaneous, physiological doses (1 γ per minute per kgm body weight) by intravenous infusion all through the experiment and for the last 10 min before its beginning.

About 0.5 ml of plasma was secured from the carotid at intervals also in these experiments. After wet ashing of the plasma sample 500 micrograms of iron were added, the iron precipitated as FeS , as described by AGNER, BONNICHSEN and one of us (1954), filtered through a perforated aluminium dish, and its activity measured.

In five experiments with mice 0.2 γ of labelled FeCl_3 was injected intraperitoneally to each of 20–50 mice, half of which were injected with 1 to 10 γ of adrenaline. The mice were killed 50 min after being injected and the ^{59}Fe content of the plasma and organs was determined.

RESULTS

a) Effect of adrenaline on the distribution of intraperitoneally injected ^{32}P in the mouse

The result of the preliminary experiment carried out with 220 mice is an absence of a significant difference between the ^{32}P content of the dry liver samples and dry muscle samples of the same weight of adrenaline injected mice and of controls, the ratio being 0.99 and 0.95. Administration of adrenaline, however, led to a 20 % depressed uptake of P^{32} by the dry femur. The plasma of the adrenaline injected mice contained at the end of the experiment, which took 15 min, 29 % ^{32}P only of that of the controls.

In the final experiments, administration of adrenaline as seen in Table 1 did not significantly influence the uptake by the liver (ratio 0.97) and only slightly that by the muscles (ratio 0.94), but it strongly depressed, by 40 %, the uptake by the femur and also the ^{32}P content of the plasma, the mean value of which was reduced by 32%, as seen in Table 1.

TABLE 1. — RATIO OF THE ^{32}P CONTENT OF THE SAME DRY WEIGHT OF ORGANS OF ADRENALINE INJECTED AND OF CONTROL MICE 15 MIN. AFTER INTRAPERITONEAL INJECTION

Ratio of ^{32}P content		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Mean value
Plasma												
Inj. ^{32}P	contr. $\times 10^{-2}$	3.48	1.54	1.905	2.08	1.03	1.81	1.595	1.27	1.58	1.0	1.729
Plasma												
Inj. ^{32}P	adren. $\times 10^{-2}$	2.85	1.08	1.51	1.342	0.728	1.06	1.09	0.775	0.787	0.735	1.196
Plasma												
Inj. ^{32}P	adren. $\times 10^{-2}$	0.82	0.701	0.794	0.645	0.71	0.585	0.685	0.61	0.498	0.735	0.678
Of plasma and liver in controls		0.897	0.517	0.696	1.01	0.883	0.745	0.855	0.562	0.801	0.591	0.756
Of plasma and bone in controls		2.2	1.931	2.19	2.78	2.0	2.28	2.17	1.871	2.21	2.35	2.198
Of plasma and muscle in controls		3.47	2.599	3.85	3.86	3.785	3.41	3.46	2.65	3.57	3.16	3.381
Of plasma and liver in adrenaline injected		0.65	0.308	0.512	0.634	0.733	0.528	0.614	0.298	0.487	0.502	0.527
Of plasma and bone in adrenaline injected		2.48	2.24	2.95	2.48	2.76	2.43	2.92	1.73	2.35	2.735	2.507
Of plasma and muscle in adrenaline injected		2.73	1.615	2.73	2.70	3.13	2.2	2.46	1.588	2.29	2.6	2.404
Plasma												
Liver	adren. $\times 10^{-2}$	0.725	0.596	0.736	0.628	0.83	0.710	0.718	0.53	0.608	0.850	0.693
Plasma												
Bone	adren. $\times 10^{-2}$	1.128	1.16	1.349	0.893	1.38	1.067	1.345	0.925	1.062	1.162	1.147
Plasma												
Muscle	adren. $\times 10^{-2}$	0.787	0.622	0.709	0.700	0.827	0.645	0.712	0.598	0.642	0.824	0.707
Liver												
Bone	adren. $\times 10^{-2}$	1.552	1.941	1.833	1.421	1.665	1.503	1.876	1.794	1.741	1.368	1.665
Liver												
Muscle	adren. $\times 10^{-2}$	1.081	1.049	0.696	1.115	1.0	0.909	0.99	1.128	1.05	0.967	1.026

It may be due to a decreased circulation rate in the bone tissue that, under the action of adrenaline, incorporation of ^{32}P into the skeleton is reduced. Furthermore, recrystallization of the bone apatite, which to a large extent is responsible for the ^{32}P incorporation, is presumably an enzymatic process and it is quite possible that adrenaline interferes with the latter.

The markedly lower ^{32}P content of the plasma of adrenaline injected mice could be due to a lower resorption rate due to the effect of adrenaline or an increased rate of efflux from the circulation. It is hardly probable that the former is the case. Adrenaline was found (1954) to decrease the resorption rate of intraperitoneally injected bicarbonate, the difference in the rate of resorption due to the presence of adrenaline manifests itself, however, in the course of the first few minutes only. We can expect phosphate to show a similar behaviour. To make sure that we are confronted with an enhanced rate of loss of ^{32}P by the plasma under the effect of adrenaline, we injected labelled phosphate intravenously into rabbits, eliminating thus the possible rôle of a resorption process.

The decrease observed in the ^{32}P content of the plasma of the adrenaline injected mouse is due to an increased rate of interchange between plasma and extravascular phosphate, and not to an increased exodus of plasma phosphate. This follows from the fact that, in our experiments with mice taking 15 min, the inorganic P content of the plasma is not influenced by the presence of adrenaline, as seen in Table 2.

PINCUS and assoc. (1933) investigated the effect of subcutaneously administered 80 microgram/kgm of adrenaline on the inorganic phosphate

TABLE 2. — INORGANIC P CONTENT OF THE PLASMA OF CONTROLS AND OF ADRENALINE INJECTED MICE

Groups of 10 mice each	Inorganic P mgm%	
	Controls	Adrenaline injected
1	5.40	5.27
2	5.08	5.03
3	4.64	6.34
4	7.10	6.38
5	6.48	—
6	5.88	6.23
7	6.38	5.50
8	5.52	6.41
9	4.70	4.05
10	9.27	8.54
11	6.73	5.85
12	5.87	6.50
Mean value	6.09 ± 1.28	6.12 ± 1.14

content of rabbit plasma. After the lapse of 15 min, they found 1% decrease only, one of 12% after the lapse of 1 hr. The observation that the inorganic P content of the plasma is reduced several hours after administration of adrenaline was reported by CORI (1930) at an early date.

Effect of adrenaline on the rate of extrusion of intravenously injected labelled phosphate from the circulation of the rabbit

Figure 1 demonstrates the rate of disappearance of the intravenously injected ^{32}P , 0.2 ml saline containing 0.1 mgm P as labelled phosphate,

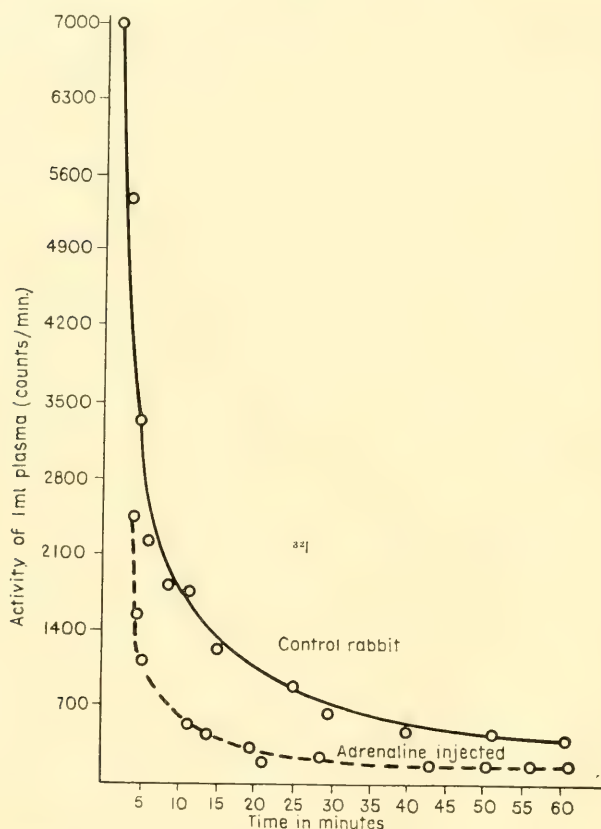


FIG. 1. Effect of subcutaneous and intravenous injection of adrenaline on the rate of extrusion of ^{32}P from the circulation of the rabbit.

from the circulation both in a control rabbit and in a sister rabbit to which 20 γ of adrenaline were administered subcutaneously 17 min before and again 26 min after ^{32}P was injected.

Several experiments yielding similar results were carried out.

The rate of disappearance of ^{32}P from the circulation is markedly accelerated in all cases investigated. The phosphate which left the circulation may remain in the interspaces into which it first penetrates, it may return into the circulation or penetrate into the tissue cells, the last mentioned process becoming more and more predominant with increasing time. The effect of adrenaline is most pronounced in the early phase of the experiment.

Effect of adrenoxyl on the rate of phosphate interchange between plasma and tissue

Adrenoxyl, the monosemicarbazone of adrenochochrome, is endowed with a marked haemostatic action. It reduces the mean bleeding time appreciably. We wished to investigate whether the rate of capillary passage can be influenced by administration of adrenoxyl. While 1 mgm of adrenoxyl administered to humans was found to reduce the bleeding time by 30% — ROSKAM (1947) — 0.41 mgm of the drug given by subcutaneous injection 1 to 3 hours before injecting the ^{32}P , and the same amount injected again intravenously to the rabbit shortly before the administration of ^{32}P , did not diminish the rate of passage of labelled phosphate through the capillary wall, as seen in Fig. 2, which indicates even a somewhat increased rate of passage.

The adrenochochrome Labaz was kindly presented to us by the Company Labaz and by Kabi A. B. and, in an investigation most kindly carried out by Prof. ULF VON EULER, was found to be free from adrenaline.

Effect of Adrenaline on the Interaction of ^{59}Fe between Plasma and Tissue

a) Disappearance of ^{59}Fe from the plasma of intraperitoneally injected mice

We tested the rate of disappearance of intraperitoneally injected labelled FeCl_3 from the plasma of the mouse. 10 mgm saline containing 0.04 microgram of Fe, and having an activity of 0.05 microcurie, were injected both to controls and to 20 min earlier subcutaneously with 10 γ adrenaline injected mice. The activity of the same volume of plasma was compared 50 min after injecting the ^{59}Fe . As seen from Table 3, adrenaline accelerates the disappearance of the ^{59}Fe from the circulation. Table 4 contains data on the ^{59}Fe from the circulation. Table 4 contains data on the ^{59}Fe uptake by the organs of controls and adrenaline injected mice. The liver of adrenaline injected mice takes up 1.9 times as much ^{59}Fe than that of the controls. In another group of experiments a corresponding ratio of 1.8 was found. As 1 ml of plasma at the end of the

experiment had an activity corresponding to 860 counts per min a minor part of the difference in the ^{59}Fe content of the organs of controls and adrenaline injected mice (620 counts) may be due to a change in their blood content.

TABLE 3. — RATIO OF THE ^{59}Fe CONTENT OF 1 GM OF POOLED PLASMA OF 10 CONTROLS AND 10 ADRENALINE INJECTED MICE

Number of experiment	Ratio of activity control: adrenaline
1	1.72
2	2.40
3	1.37

TABLE 4. — EFFECT OF ADRENALINE ON THE UPTAKE OF INTRAPERITONEALLY INJECTED ^{59}Fe BY THE POOLED ORGANS OF 10 MICE WEIGHING 166 (C) AND 176 (A) GM

Organ	Fresh weight in gm	Dry weight in gm	Counts/min given by total tissue	Percentage change due to adrenaline
Liver C	6.8907	1.9105	4560	+ 90.7
Liver A	7.5583	2.1985	8690	
Spleen C	0.8765	0.2106	1685	— 49.9
Spleen A	0.5586	0.1361	845	
Lungs C	1.2635	0.2831	711	+ 21
Lungs A	1.7735	0.3754	860	
Kidneys C	1.5907	0.4243	514	+ 52
Kidneys A	2.0982	0.5868	795	
Muscles C	2.2586	0.5704	800	— 61.7
Muscles A	2.9593	0.7494	306	

C denotes Controls. — A denotes adrenaline injected mice.

b) Disappearance of ^{59}Fe from the circulation of rabbits into which labelled iron- β -globuline containing plasma was injected

FLEXNER and assoc. (1948) have shown that after transfusing plasma, to which labelled FeCl_3 was added, *in vitro* to a guinea pig, the activity of the plasma decreases first with a half-time of about 20 min but, after the lapse of 20–30 min, a further decrease in the plasma activity takes place with a half-value of about 3 hr. The initial rapid disappearance of plasma activity is due to the exodus of ^{59}Fe which had no opportunity to combine with β_1 -globulin, while the remaining iron which had

opportunity to combine with that protein escapes at a much reduced rate.

In a preliminary experiment we injected into the ear vein of a donor rabbit 84 γ of labelled iron and transfused 18 ml of whole blood after the lapse of 1 hour to a sister rabbit. This procedure proved not to be satisfactory, half of the transfused ^{59}Fe disappearing from the circulation

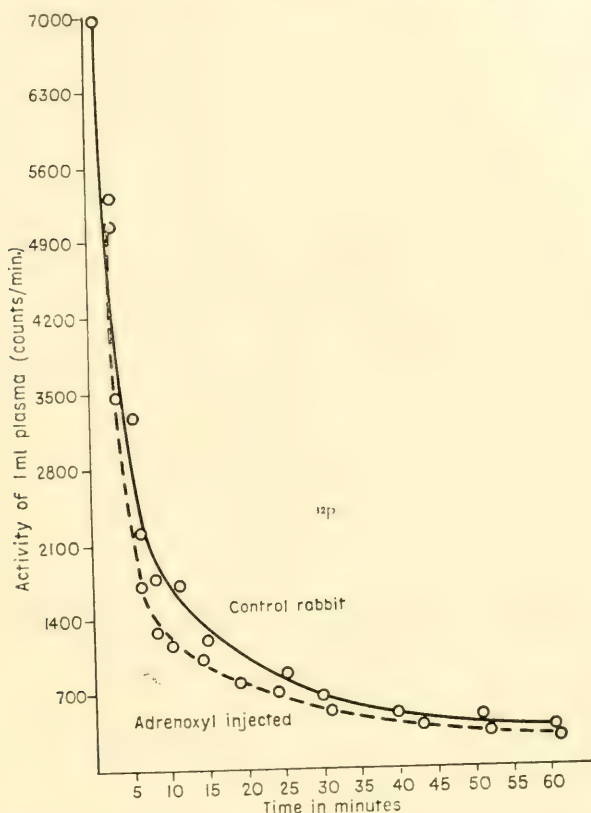


FIG. 2. Effect of subcutaneous injection of adrenoxyl on the rate of extrusion of ^{32}P from the circulation of the rabbit.

already in the course for 27 min, a further half in the course of 60 min. We then injected the donor rabbit with 1 γ of iron only having an activity of 0.9 micro C, and after the lapse of 1 hr transfused to a sister rabbit 10 ml of the plasma of the donor, securing at intervals plasma samples from the receptor. The result of this and a second similar experiment is seen in Fig. 5.

The experiment was then repeated with receptor rabbits to each of which 20 γ of adrenaline were administered subcutaneously 52 min and 33 min before transfusing the activity plasma.

In another experiment we administered a physiological dose of adrenaline to the recipient rabbit, infusing all through the experiment 0.5 ml of saline per min containing 1 γ of adrenaline per kgm body weight.

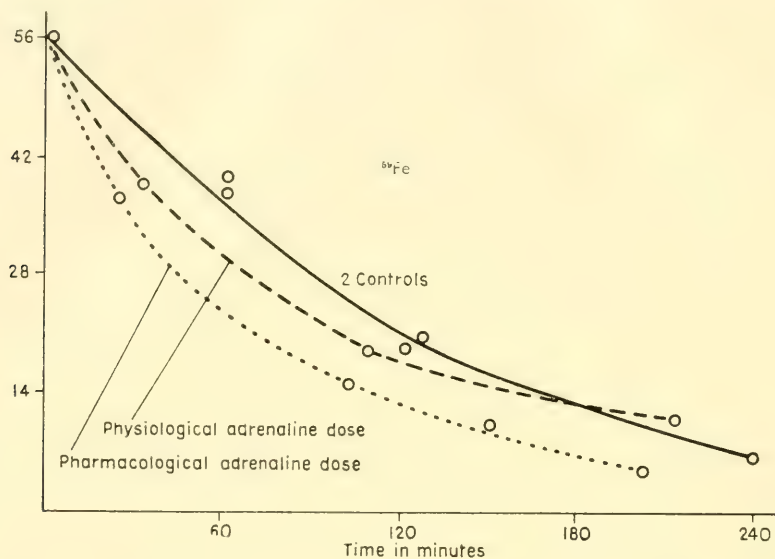


FIG. 3. Effect of injection of adrenaline on the rate of extrusion of ^{59}Fe from the plasma of the rabbit.

In both experiments a marked increase of the disappearance of ^{59}Fe from the circulation was observed under the action of adrenaline, the effect of a pharmacological dose being the more pronounced one.

DISCUSSION

Following injection of labelled ions into the jugular vein there is a large arteriovenous concentration difference which decreases exponentially with time (PAPPENHEIMER, 1950; SCHLOERB, 1950). It is conceivable that adrenaline accelerates this decrease and thus accelerates the extrusion of the labelled ions from the vascular bed. The rate of extrusion may be determined by the rate of blood flow which is accelerated by small doses of adrenaline. The fact that adrenaline influences markedly the rate of passage of intravenously injected ^{32}P as phosphate or ^{59}Fe circulating as β -globulin into the extravascular space is not necessarily to be interpreted as due to a change produced in the permeability of the capillary wall. Phosphate which passes from the vascular bed into the interspaces may repeatedly return into the former and escape again. If, however, it took its way from the interspaces into the tissue cells,

the chance of returning is a rather small one in view of the large phosphate pool of tissue cells. An escape of the ^{32}P into the cells will thus reduce the probability of its return from the interspaces into the vascular bed and hence will ultimately accelerate the rate of exodus of ^{32}P from the plasma.

That injected ^{42}K leaves the plasma at a much more rapid rate than injected ^{24}Na , observed in the earliest experiment of this type (HAHN, *et al.* 1941), and interpreted as due to the large potassium pool of the tissue

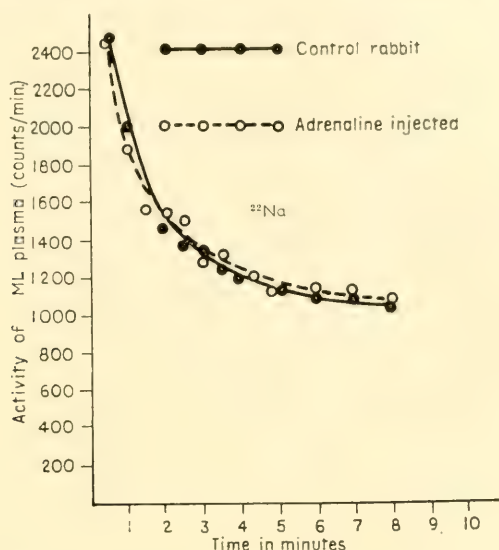


FIG. 4. Effect of injection of adrenaline on the rate of extrusion of ^{22}Na from the circulation of the rabbit

cells in contrast to their restricted sodium pool. Should adrenaline accelerate the rate of interchange between the phosphate of the interspaces and that of the tissue cells, this may explain its effect on the rate of exodus of ^{32}P from the vascular bed. Doses of adrenaline as applied by us are known to increase the oxygen consumption (CARR, 1934; LUNDHOLM, 1949).

In the case of sodium the interchange between interspaces and tissue cells having a restricted importance only, as the incorporation of Na^{24} into the skeleton and cartilage, which harbours most of the not extracellular sodium, takes some time, adrenaline should not much accelerate the exodus of ^{24}Na in experiments taking about 1 hour only.

It is of interest to note that while from the sodium content of the ear cartilage of the rabbit follows an apparent extracellular volume of that organ amounting to 96.6% of its weight, we found 10 min following the injection of radiosodium into the circulation of the rabbit a ^{22}Na content of the ear cartilage which corresponds to an extracellular space

of 5% only. Thus, interchange between plasma sodium and the sodium of the ear cartilage is a slow one. Injection of adrenaline increased the last mentioned figure to 10%.

We could not observe any acceleration of the exodus of ^{24}Na from the circulation under the action of adrenaline (cf. Fig. 4), in some of our experiments (see Fig. 5) even a decrease in the rate of extrusion of radio-sodium took place. An observation which may be taken to support the interpretation of the accelerated disappearance of ^{32}P and of ^{59}Fe of the

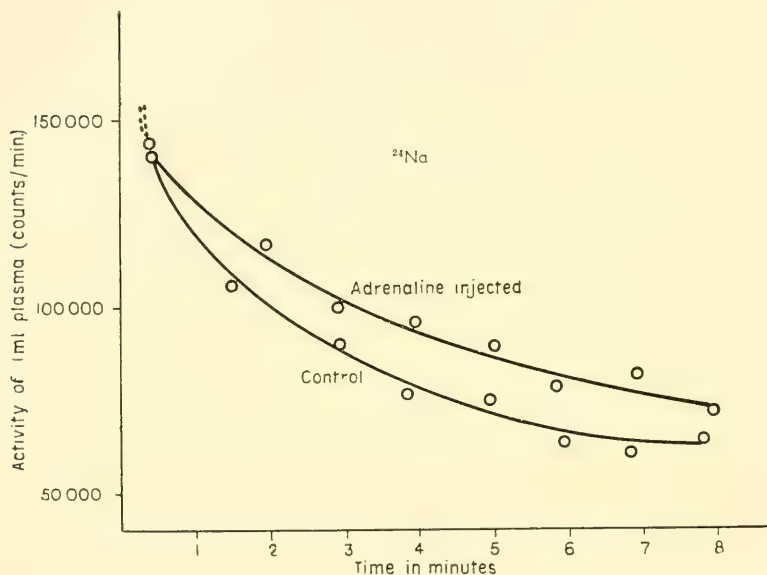


FIG. 5. Effect of injection of adrenaline on the rate of extrusion of ^{24}Na from the circulation of the rabbit.

plasma under the effect of adrenaline as to be due to an enhanced interaction rate between the phosphate resp. iron present in the tissue cells and that present in the interspaces. DOBSON and his associates (1953) found that the removal rate of sodium injected into an artery is greatly accelerated by a systematic administration of adrenaline.

The additional ^{59}Fe given off by the plasma under the effect of adrenaline was found by us to find its way to a large extent into the liver in which presumably an enhanced iron incorporation takes place under the effect of adrenaline. Adrenaline was observed to produce hypoferemia in dog by GUBLER (1950) and in human by BATEMAN (1952). Adrenaline was found to increase the oxygen consumption (CARR, 1934; LUNDHOLM, 1949).

From the activity of the injected plasma and of that of 1 ml plasma secured after the lapse of 3 min follows a plasma content of the rabbit

amounting to 80 ml. As the iron content of the plasma amounts to 1.8 γ per ml and the half-life of the plasma ^{59}Fe to 1.5 hr. the amount of ^{59}Fe turned over in the course of 1 hr works out to be
$$\frac{0.693 \cdot 1.8 \cdot 80}{1.5} = 66 \gamma.$$

Injection of a pharmacological dose of adrenaline increases thus the amount of plasma iron turned over per hour to 130 γ .

It is of interest to compare this increase with a decrease in the rate of plasma turnover due to total irradiation of the rabbit. As seen in

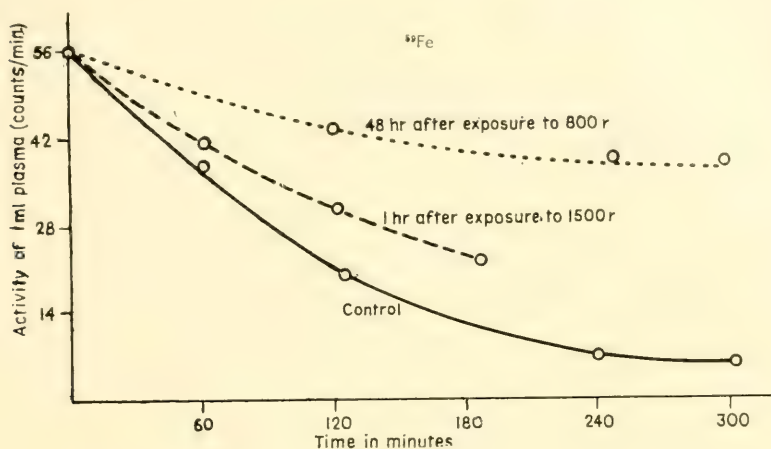


FIG. 6. Effect of irradiation with Roentgen rays on the rate of extrusion of ^{59}Fe from the plasma of the rabbit.

Fig. 6 measured 1 hr after irradiation with a high dose of 1,500 r the amount of plasma iron turned over in the course of 1 hr. decreased to 42 γ . Measured 48 hr after irradiation with 800 r only the decrease is much more pronounced the amount turned over being less than 30 γ .

The difference in the rate of plasma iron turnover shortly and 2 days after exposure is presumably at least partly due to the fact that shortly after irradiation orthochromatic and even polychromatic erythroblasts are present in marrow which were not destroyed by exposure to radiation and take up ^{59}Fe previous to their removal into the circulation. After the lapse of several hours these erythroblasts matured and were given off to the circulation but were not renewed in the marrow as irradiation suppresses the formation of new cells and even destroys such as are in an earlier phase of their maturation.

Experiments in which massive doses of adrenoxyl were administered to rabbits did not reveal any tightening of the capillary wall to the passage of ^{32}P , a small increase in the rate of exodus was even observed. Adrenoxyl influences thus the interchange between the vascular and

extravascular ^{32}P in a similar sense, though to a much restricted extent, than does adrenaline.

Adrenaline is known to bring about a violent increase in the outflux of sodium through surviving frog skin, while to increase moderately only the influx of sodium (USSING, 1952). A third compartment in which sodium could accumulate was absent in these experiments.

GEMZELL and SAMUELS (1950) investigated the effect of ACTH on the ^{32}P content of the plasma of rats injected intraperitoneally with radiophosphate. They found the administration of the adrenocorticotrophic hormone to diminish with 15% both the ^{32}P and ^{31}P content of the plasma 50 min after the injection of radiophosphate. No increase in the ^{32}P content of the liver was observed. In hypophysectomized rats the ^{32}P content of the plasma, however, is conserved for a longer time than in controls, as observed by GESCHWIND and assoc. (1950).

Summary

Administration of adrenaline to mice before intraperitoneal injection of ^{32}P leads to a markedly increased rate of passage of the resorbed ^{32}P from the plasma into the tissues.

The rate at which ^{32}P leaves the circulation of the rabbit after intravenous injection is accelerated to twice its normal value if a pharmacological dose of adrenal was administered previously.

Massive doses of adrenoxyl lead to a slightly increased rate of exodus of the intravenously injected ^{32}P from the circulation of the rabbit.

After intraperitoneal injection of $^{59}\text{FeCl}_3$ to mice ^{59}Fe leaves at a much enhanced rate the plasma after administration of adrenaline. Much of the ^{59}Fe is recovered in the liver.

Labelled iron of $^{59}\text{Fe}-\beta_1$ -globulin transfused with the plasma of a donor rabbit to a recipient rabbit, leaves the circulation if a pharmacological dose of adrenaline is administered at a twice accelerated rate.

In contrast to administration of adrenaline, exposure to X-rays strongly decreases the exodus of ^{59}Fe .

Adrenaline does not accelerate the rate of exodus of intravenously injected labelled sodium from the circulation of the rabbit.

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62. EFFECT OF IRRADIATION ON HEMIN FORMATION

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Irradiation with Roentgen rays was found to depress very appreciably the rate of extrusion of ^{59}Fe bound to β_1 -globulin in the circulation of the rabbit, the depression being much more pronounced 2 days after irradiation than shortly after exposure. It is suggested that erythrocytes of the bone marrow which have not yet completed their hemoglobin content are radioresistant and continue to incorporate ^{59}Fe in the bone marrow of the irradiated animal. Correspondingly, the effect of exposure to radiation on the rate of extrusion of ^{59}Fe from the plasma gets strongly pronounced only after the lapse of several hours.

A radiation dose which reduces the incorporation of ^{59}Fe into the hemoglobin to 30% of that of controls does not diminish the rate of incorporation of ^{59}Fe into cytochrom b of the liver of guinea-pigs.

THE study of iron metabolism can be approached in different ways, a very profitable one being the study of the fate of plasma iron. Iron utilized for hemoglobin formation has to pass the plasma, so iron coming from and going to the various organs in which it is present as a constituent of ferritin, hemosiderin, myoglobin, cytochrom, catalase or other compounds.

FLEXNER *et al.*⁽¹⁾ injecting labelled ferric chloride into the circulation of guinea-pigs observed a rapid initial disappearance of the radioactive iron from the circulation followed by a slower process. They interpreted the first process to be due to the disappearance of inorganic iron, the second to iron bound to proteins. These early experiments thus brought out already the difference between the circulating "physiological" iron and the plasma foreign iron injected into the circulation. The physiological plasma iron is bound to the β_1 -globuline of the plasma which amounts to 3%, of the plasma proteins. This protein fraction, the transferrin, is identical with Cohn's fraction IV—7 as shown by HOLMBERG and LAURELL⁽²⁾, by LAURELL⁽³⁾ and others. That the albumine fraction of the plasma proteins does not fix iron, was recently demonstrated by WUHRMANN and JASINSKI⁽⁴⁾ by combining paper chromatographic with radioactive measurements. From the spots of the paper diagram of the plasma proteins from a rabbit 10 minutes previously injected with

labelled ferric chloride only that due to β_1 -globulin was found to be radioactive.

How the turnover rate of the circulating plasma iron is influenced by interference with hemopoietic processes going on in the human and animal organism was investigated in numerous cases by John LAWRENCE and his colleagues and several others.

Being interested in the problem of radiation anemia we determined the rate of disappearance of labelled plasma globulin from the circulation of rabbits exposed to Roentgen rays and the incorporation of ^{59}Fe into the circulating hemoglobin and into marrow hemin of guinea-pigs and rabbits. The effect of irradiation on the incorporation of ^{59}Fe into the red corpuscles of the rabbit and of the rat was previously investigated by HUFF, HENESSY and their associates^(5,6).

In view of the marked effect of irradiation on the incorporation of ^{58}Fe into hemoglobin we wished to know if hemins formed in another *milieu* than the bone marrow are affected by irradiation as well. We investigated therefore the effect of exposure to radiation on the incorporation of ^{59}Fe into the iron containing enzymes of the liver and into cytochrome c and myoglobin of the muscles. In this note results obtained in the investigation of cytochrome b (Strittmatter⁽²³⁾) of the liver and that of myoglobin are communicated.

EXPERIMENTAL

In 21 experiments with guinea-pigs an aggregate number of 220 animals, weighing 450–700 gm, were injected intraperitoneally with 0.2–0.4 ml of saline containing 2 to 7 μgm of with ^{59}Fe labelled iron of $1/2$ – $1/4$ μCurie activity. Half of the guinea-pigs was exposed to X-rays (160 kV, 43 r per min for 10 to 33 minutes). Injection took place 15 to 300 min following exposure. No food was administered during the experiment.

The animals were killed 4 to 48 hrs later, their livers homogenized in sucrose and fractionated as described by HOGBEIN and SCHNEIDER. Ferritin was extracted both from microsomes and from the supernatant, cytochrom b hemin from microsomes (LOFTFIELD⁽⁷⁾) and catalase from the supernatant. Total iron, cytochrome c and myoglobin iron were furthermore isolated from the muscles making use of the method of THEORELL and ÅKESON⁽⁸⁾.

Ferritin was precipitated from the supernatant by addition of half volume of ammoniumsulfate. The precipitate was taken up in water and repeatedly fractionated with ammoniumsulfate. The precipitate was then taken up in water, heated to 70° C for a few minutes and centrifuged after cooling. The supernatant ferritin was found to contain 17 to 20% iron.

The microsomes were homogenized in water, the pH adjusted to 5 and centrifuged after the lapse of 30 minutes. The precipitate was once more homogenized and washed with water. All the microsome ferritin was found to be in the water phase. The insoluble particles contained the cytochrome b and some protein-bound iron. The cytochrome b hemin was extracted from this fraction with a mixture of acetone and HCl (10 ml 20% HCl in 1 liter of acetone). The acetone

was evaporated *in vacuo*, the precipitated hemin washed with 1 N HCl and dissolved in ether. The ether was evaporated and the hemin combusted with sulfuric acid and hydrogen peroxide. The determination of iron was carried out by a colorimetric method using the sulfosalicylic acid complex.

An aliquot of the solution was used for colorimetric determination of the iron content, to another aliquot iron chloride was added bringing its iron content up to 500 μg . The iron was precipitated as FeS and filtered through a perforated aluminium dish covered with filter paper as described by AGNER *et al.*⁽⁹⁾ The aluminium dish was then placed under the window of a Geiger counter. An aliquot of the solution which was injected was treated in the same way. The data obtained permitted to calculate the relative specific activity of the iron fraction (activity per mgm iron in arbitrary units) and also the absolute specific activity (percentage of injected ^{59}Fe present in 1 mgm of iron).

In our experiments on the iron turnover of the plasma 5–10 μgm of iron having an activity of about 2 μCurie were injected into the ear vein of a rabbit. After the lapse of about 1 h a few ml of the plasma of this rabbit, which now contained all ^{59}Fe present in the plasma as β_1 -globulin, was injected into the jugular vein of a sister rabbit. At intervals blood samples were taken from the carotic vein of this rabbit, the heparinized blood centrifuged, the plasma combusted as described above, 500 μgm carrier iron added and the iron precipitated as sulphide as described above. 2–3 kgm rabbits were applied in these experiments, in which the effect of irradiation on the formation of labelled hemoglobin and labelled hemin of the bone marrow was investigated.

RESULTS

a) Cytochrome b

The data obtained for the ratio of the specific activities of cytochrome biron (STRITTMATTER⁽²³⁾) and also of microsome ferritin iron of irradiate and control guinea-pigs are listed in Table 1. Out of 13 ratios listed in Table 1, one is as low as 0.77, but even this ratio is by about 250% higher than that obtained for hemoglobin iron, which was found to be 0.3, corresponding to a depression of ^{59}Fe incorporation into hemoglobin in the irradiated guinea-pig to $1/3$ of that into the hemoglobin of controls.

Not only does exposure of guinea-pigs to large doses of Roentgen rays not diminish the rate of incorporation of the ^{59}Fe into cytochrome b but as seen in Table 1 promotes it. The increased incorporation of ^{59}Fe does, however, not or not mainly indicate an increased rate of formation of cytochrome b. It is, at least to a large extent, due to the change in sensitivity of the radioactive indicator. A larger part of the plasma iron takes its way into the bone marrow and is applied to hemoglobin synthesis, a minor part into the liver and other organs. The labelled iron atoms which leave the plasma are replaced by inactive ones coming from the organ depots and to a minor extent from the intestine. Irradiation upsets distribution. Incorporation into hemoglobin is strongly reduced which results in an increased life-time of ^{59}Fe in the plasma which in turn leads

to a change in the sensitivity of the radioactive indicator. The increase in the iron content of the plasma of the irradiated animals (SCHUCK,⁽¹⁰⁾ LUDEWIG⁽¹¹⁾) remains behind the increase of its ⁵⁹Fe content and correspondingly the sensitivity of the radioactive indicator decreases. Thus we can expect more ⁵⁹Fe to reach the liver in the exposed animals than in

TABLE 1. — EFFECT EXPOSURE TO 500—1 400 R ON THE INCORPORATION OF ⁵⁹Fe INTO THE CYTOCHROME b OF THE LIVER OF GUINEA-PIGS. EACH FIGURE IS OBTAINED BY POOLING 5 LIVERS

Time between injection of ⁵⁹ Fe and removal of the liver in hr	Ratio of spec. activ. of cytochrome b iron of irradiated and control animals	Ratio of spec. activity of microsome ferritin iron of irradiated and control animals
4	2.52	1.62
4	0.77	1.12
4	0.96	1.05
16	1.17	0.91
17	1.48	1.35
17	0.88	1.16
17	0.92	1.06
17	1.90	1.39
17	1.15	1.40
48	1.12	0.94
48	1.16	1.19
48	1.25	1.81
48	0.94	2.13
Mean value	1.25 ± 0.036	1.32 ± 0.010

the controls. If this line of thought is correct we must expect the ratio of the specific activity of the iron of exposed and control animals, which for cytochrome b was found to be 1.3, to be about 1.3 for other liver fractions as well (as far as their rate of formation is not influenced by irradiation). As seen from Table 1 this is almost the case for microsome ferritin, 1.32 being found for the corresponding ratio.

ELMLINGER *et al.*⁽¹²⁾ found in normal humans about 55% of the iron which passed the plasma to be utilized in red corpuscle formation. The rest takes its way into the other organs. Should the last mentioned inflow be unilateral, the iron content of these organs would increase already in the course of 120 days with about 2 gm which is more than the total iron depot of a man which amounts to about 1.6 g (HASKINS⁽¹³⁾). We have therefore to conclude that the flow of iron from the plasma into the depot organs goes hand in hand with an outflow of an even larger amount of iron from the depots into the plasma.

A different line of thought supports this view. Hemoglobin iron is almost quantitatively reutilized for hemoglobin formation as shown by HAHN *et al.*⁽²⁴⁾. We injected labelled red corpuscles of a donor rabbit to an acceptor rabbit and after the short interval of only 5 hours found 90 times more ^{59}Fe in the liver than in the spleen of the thoroughly perfused animals, the gall containing 2/3 of that of the last mentioned organ. The reutilization of this iron for hemoglobin formation necessitates its passage through the plasma to the marrow. Thus a continuous flow of iron from the liver (and presumably other organs responsible for the phagocytosis of red corpuscles) to plasma has to take place. In the course of 120 days (the time necessary to replace the circulating human red corpuscles) about 2 gm of iron has to pass from the depot organs to the bone marrow. The corresponding figure in the rabbit in the course of 50 days is about 0.1.

Effect of irradiation on ^{59}Fe incorporation into hemoglobin

As to be expected and also shown by HUFF⁽⁵⁾, HENNESSY⁽⁶⁾ and their associates, irradiation strongly depresses the incorporation of ^{59}Fe into hemoglobin. Our results obtained with guinea-pigs injected within 6 hours after exposure and killed 17 hours later are seen in Table 2.

In each of the experiments Nos. 9 to 16 the blood of 10 guinea-pigs, in Nos. 21 and 22 of 20 animals was pooled.

Incorporation of ^{59}Fe into hemoglobin was thus reduced by irradiation in these experiments to 30% of that of controls.

TABLE 2. — EFFECT OF IRRADIATION
WITH 500—1300 R ON THE INCORPORATION OF ^{59}Fe INTO HEMOGLOBIN
OF GUINEA-PIGS

Number of experiment	Ratio of spec. activ. of hemoglobin iron of irradiated and control guinea-pigs
9	0.20
10	0.17
12	0.36
13	0.72
14	0.25
15	0.21
16	0.29
21	0.24
22	0.28

Mean value : 0.298 ± 0.158

Delay of the effect of irradiation on the incorporation of labelled iron into hemoglobin

Red corpuscles are very radioresistant. We need very massive doses to achieve hemolysis and a dose of 4000 r for example as shown by SHEPPARD⁽¹⁴⁾ does not influence the rate of intrusion of ^{42}K in the red corpuscle though it does to some extent weaken the mechanism responsible for the concentration of potassium in the red corpuscles. Dog blood exposed to a dose of 200,000 r does not hemolyse as observed by NIZET *et al.*⁽²²⁾

The radioresistance of circulating red corpuscles suggests that the not fully completed red corpuscles in the bone marrow are radiation resistant as well and continue even in the exposed animal to complete their hemoglobin content. In the bone marrow of swine exposed to radiation of the Bikini explosion often only fat cells and a few clumps of erythrocytes were found⁽²⁵⁾.

The above conclusion is supported by the observation that the uptake of ^{59}Fe or ^{14}C by the hemoglobin of red corpuscles *in vitro* is not reduced by irradiating the animal before securing the blood sample. Reticulocytes and other types of incomplete red corpuscles can complete their hemoglobin content *in vitro*. Not only is the ^{14}C incorporation *in vitro* into the blood corpuscles of animals not smaller than into those of controls, it is even markedly enhanced as shown by NIZET *et al.*⁽¹⁵⁾ who investigated the uptake of ^{14}C by the dog. RAUNTANAN and one of us made similar observations when studying incorporation of ^{59}Fe *in vitro* into the red corpuscles of the hen which was found to be increased in extreme cases up to 300% if the hen was 18 hours previously exposed to a dose of 1000 r. Irradiation induces presumably the bone marrow to release some of the red corpuscles in an earlier stage of their maturation. NIZET *et al.* have furthermore shown that irradiation *in vitro* produces changes in the plasma which are favourable to ^{59}Fe incorporation into the red corpuscles.

These considerations support the conclusion that as far as red corpuscles in the advanced stage of their maturation are present in the bone marrow, a completion of their hemoglobin content takes place even in the exposed organism and that some of the ^{59}Fe administered shortly after irradiation will be utilized by the bone marrow. With increasing time the completed red corpuscles being discharged into the circulation or wiped out in the exposed organism, the utilization of ^{59}Fe by the bone marrow will diminish and after the lapse of about 1 day may almost cease. As the non-utilization of labelled plasma iron by the bone-marrow leads to a depressed turnover rate of the plasma iron, this depression is to be expected not to be shown to its full extent already shortly after irradiation but only at a later date. That this is the case is demonstrated by Fig. 1 and the data of Table 2.

As seen in Fig. 1, the effect of irradiation with such a high dose as 1500 r influences the rate of extrusion of ^{59}Fe from the plasma, when measured 1 hour only after exposure, is much less than an irradiation with 800 r only, when the rate of extrusion is measured 2 days after irradiation.

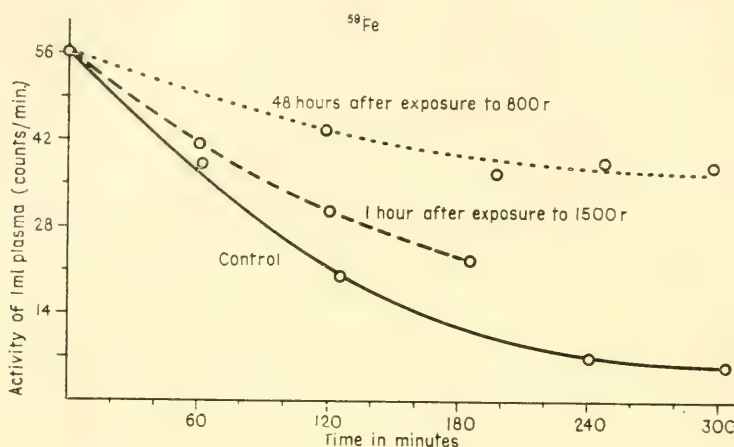


FIG. 1. Effects of exposure to Roentgen rays on the rate of extrusion of circulating labelled plasma iron from the circulation of rabbits.

TABLE 3. — INCORPORATION OF INTERPERITONEALLY INJECTED ^{59}Fe IN THE COURSE OF 5 HOURS INTO THE HEMOGLOBIN OF CONTROLS AND WITH 500 R IRRADIATED RABBITS. C = CONTROL. R = EXPOSED

Time in hours between exposure and injecting of ^{59}Fe	Relative ^{59}Fe content			
	Plasma		Hemoglobin	
	C	R	C	R
0	100	100	100	100
0.5	100	118	100	68*
17	100	124	100	56
48	100	223	100	3.2
72**	100	447	100	17.3

* For the bone marrow hemin the corresponding figure was 43.

** Data obtained by Huff *et al.*,⁽¹⁰⁾

When injecting rabbits weighing 600 gm intraperitoneally with labelled FeCl_3 48 hours after exposure to 500 r, we observed 5 hours after injection the ^{59}Fe content of the plasma to be 120% higher than that of the control. In experiments in which 500–900 gm rabbits were injected intraperitoneally within 1/2 hour after irradiation with 5 μgm labelled iron as FeCl_3 and killed 3 hours later, the ^{59}Fe content of the plasma was increased by 6% only. Further data on the effect of time which elapsed after

exposure to irradiation on the ^{59}Fe content of the plasma is seen from Table 3. This table also contains data on the incorporation of ^{59}Fe into hemoglobin, which is much more markedly depressed under the effect of irradiation after the lapse of 48 hours than after that of 1 hour. In these experiments $5\text{ }\mu\text{g}$ of iron as FeCl_3 were interperitoneally injected to each rabbit. Similar observations on the effect of irradiation on the incorporation of ^{59}Fe into the red corpuscles were made by HEXNESSY and HUFF⁽⁶⁾.

These results support the conclusion arrived at that for some time after irradiation the completion of the hemoglobin content of the red corpuscles is still going on in the marrow.

b) Muscle myoglobin

In view of the fact that the chemical composition of myoglobin closely resembles that of hemoglobin — they are differing only as to their degree of polymerisation — it was of great interest to investigate the effect of exposure to irradiation on the incorporation of ^{59}Fe into the myoglobin of guinea pigs extracted from their muscles. The method of purification was that described by THEORELL and ÅKESON⁽⁸⁾. The myoglobin obtained was practically free from hemoglobin as revealed by a spectroscopic investigation of the CO-myoglobin. In 4 experiments in each of which 10 female guinea-pigs having a weight of 450–550 gm were injected 6 hours after exposure to 1400 r with $3\text{ }\mu\text{gm}$ of labelled iron as sodium iron citrate and killed 18 hours later and 10 controls were treated in the same way, the following rel. specific activity figures were obtained for the myoglobin and hemoglobin iron of controls and exposed animals.

Under effect of exposure the ^{59}Fe incorporation into myoglobin was thus reduced to 1/2, that into hemoglobin to 1/4 of that of the controls. In the 2 last experiments we determined the effect of exposure on the

TABLE 4. — EFFECT OF IRRADIATION ON THE INCORPORATION OF ^{59}Fe INTO MYOGLOBIN AND HEMOGLOBIN OF GUINEA-PIGS. CONTROL = C, EXPOSED = R

No. of expt.	Rel. spec. activity* of myoglobin		Ratio $\frac{C}{R}$	Rel. spec. activity of hemoglobin		Ratio $\frac{C}{R}$
	C	R		C	R	
1	0.098	0.042	2.3	0.236	0.047	5.0
2	0.034	0.026	1.3	—	—	—
3	0.476	0.215	2.2	2.19	0.48	4.6
4	0.415	0.164	2.6	2.44	0.68	3.6

* Count per μgm Fe.

specific activity of the total iron present in the muscles as well and found it, as to be expected increased under the effect of exposure, in the third experiment from 0.714 to 0.810, in the fourth one from 0.687 to 1.04.

DISCUSSION

Hemoglobin is one of the comparatively few molecular constituents of the adult organism that is formed in close connection with cell division. The latter being very susceptible to the effect of ionising radiation, exposure to radiation is bound to interfere with hemoglobin formation as well. Furthermore, marrow cells are radiosensitive and exposure to radiation may lead to destruction of marrow cells. Irradiation of rats with 400 r was found to lead to a decrease in the total number of marrow cells to almost one half of its normal value in the course of the first day following exposure (BRECHER⁽¹⁵⁾) and the chemical composition of the bone marrow was found markedly influenced. LUTWAK-MANN⁽¹⁷⁾ found 3 hours only after total body exposure to a dose of 1500 r the labile acid-soluble P of the bone marrow of rabbits to be reduced by 30%, that of DNA and RNA phosphorus by 20 and 16%. The total nucleic acid P of the marrow of the to 500 r exposed rat amounted to only half of that of controls. Mandel *et al.*¹⁸ report the bone-marrow of rats exposed to 500 r to show after the lapse of 26 hours a by 50% reduced PNA content, the DNA being reduced to 30% of that of the controls after the lapse of 2 days. Thus radiation anemia is at least partly due to the fact that hemoglobin is formed in close connection with the cell division and also that it takes place in the radiosensitive marrow cells.

ALTMANN *et al.* (RICHMOND⁽¹⁹⁾, STOKINGER⁽²⁰⁾) in their very extended studies found incorporation of ¹⁴C into globin and hemin to be influenced at a very different rate by irradiation. Incorporation of ¹⁴C into hemin of the exposed animals was greatly depressed, whereas globin was affected to a considerably smaller extent. From this finding it does not necessarily follow that it is not the *milieu* of its formation, the bone marrow, which is responsible for the radiation sensitivity of the formation of hemoglobin.

We found no interference with the formation of cytochrome b in the liver of the strongly irradiated guinea-pig. In this case heme formation is thus not radiosensitive. We found, however, interference with formation of myoglobin.

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63. HAEMOGLOBIN PRESENT IN THE NUCLEAR FRACTION OF THE LIVER

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The nuclear fraction of 1 gm of rat and rabbit liver contains 0.34 and 0.58 mgm of in aqueous medium non-extractable haemoglobin. The corresponding figure for the nuclei of the red corpuscles of 1 ml of hen's blood is 0.9 mgm.

The haemoglobin content of the nuclear fraction of 1 gm of foetal rabbit liver is 100 times as large as that of 1 gm of maternal liver. The ratio of their ^{59}Fe content 11 to 17 h after labelling the maternal plasma iron, works out to be 100.

One day or more after exposure of the rat to 500 r, incorporation of ^{59}Fe into the non-extractable haemoglobin of the liver nucleus fraction is strongly depressed.

As mentioned in a previous note (BONNICHSEN *et al.*⁽¹⁾) the nuclear fraction of the liver of the guinea pig contains small amounts of haemoglobin not extractable by saline or other aqueous solutions. This paper contains data on the incorporation of ^{59}Fe into this fraction isolated from the liver of adult rats and rabbits and of that of the rabbit embryo. This fraction was furthermore located in the nuclei of the erythrocytes of the hen.

That in the basophilic erythroblasts of human and rat bone marrow the site of haemoglobin synthesis is primarily the nucleus, was repeatedly suggested. The methods applied in these investigations were ultraviolet absorption microspectroscopy and cytochemical staining procedures⁽²⁻⁹⁾.

EXPERIMENTAL

In 15 experiments with male rats an aggregate number of 173 animals, weighing between 150 and 280 gm were injected intraperitoneally with 0.25 ml of a 3.8% ammonium-citrate solution containing 0.5—12 μgm of with ^{59}Fe labelled iron of 0.1—12 μC activity. Half the number of rats was exposed to 150 r — 500 r of X-rays. Injection took place 15 min to 5 days after exposure.

The animals were killed from 2 to 48 hr after injection. The livers were perfused first with 0.145 M NaCl and then with 0.25 M sucrose containing 0.018 M CaCl_2 . The weighed livers were homogenized in 9 vols. of 0.25 M sucrose — 0.0018 M CaCl_2 . The further procedure was carried out according to HOGEBOM *et al.*⁽¹⁰⁾

The nuclear fraction isolated by this procedure was cytologically inhomogeneous. The fraction contained about 60—80% of the cell nuclei, it also contained erythrocytes, connective tissue, residual intact cells and free mitochondria.

In order to haemolyse the erythrocytes, the fraction was homogenized with 10 vols. distilled water and allowed to stand several hours. This last step was repeated at least three times more.

After clearing the suspension of nuclei obtained in this way by adding 3% of deoxycholate, the haemoglobin bands were steadily visible in the handspectroscope. The CO-band was that of haemoglobin. The pyridine-haemochromogen band was located at $557\text{ m}\mu$.

The haemin was extracted with a mixture of acetone and HCl (10 ml 20% HCl in 1 l. of acetone). After filtration, the acetone was evaporated *in vacuo*. The haemin was crystallized twice from conc. acetic acid, and the crystals washed with 1 N HCl. The haemin was then combusted and the solution analyzed as described by BONNICHSEN *et al.*⁽¹¹⁾

In order to know in which part of the above nuclear fraction the non-extractable haemoglobin is located, we separated the fraction, prior to haemolysing the red cells with distilled water, in the counter-streaming centrifuge of LINDAHL⁽¹²⁾ in five fractions containing particles of different size and different specific gravity. The fractions were examined with the phase-contrast microscope and with the handspectroscope. Thereafter, the specific activity of their haemin was determined. The result of one of these experiments is seen in Fig. 1.

In five of the above experiments the liver nuclei have been isolated both in aqueous medium and in organic solvents of low polarity according to the methods described by DOUNCE *et al.*⁽²³⁾ and ALLFREY *et al.*⁽¹⁴⁾ The nuclear preparations obtained in this way were treated and analysed as described above. No difference was found in the properties of the non-extractable haemoglobin of the nuclear fraction prepared either in aqueous or in organic medium.

The liver ferritin was prepared as previously described by LOFTFIELD *et al.*⁽¹⁵⁾

In experiments with 2–3 kgm rabbits 8 animals were investigated. Their plasma was labelled with ^{59}Fe as described by EHRENSTEIN *et al.*⁽¹⁶⁾ and reinjected. The animals were killed 3–24 hr after injection.

Simultaneously, in all our experiments haemin of the circulating haemoglobin was analysed as well.

In two experiments with hens 100 ml of hen blood were incubated *in vitro* for 3 h at 37°C with 2 ml of 3.8% ammonium citrate solution containing $2\text{ }\mu\text{gm}$ labelled iron of $25\text{ }\mu\text{C}$ activity. The plasma was centrifuged off and the red cells washed four times with 5 vols. or more of isotonic saline.

The erythrocyte nuclei were prepared according to HOGBOOM *et al.*⁽¹⁰⁾ However, as the red cells are not sufficiently broken down by the homogenization procedure usually employed for the disintegration of other tissues, we haemolysed the cells by freezing at -20°C and thawing them three times or more, suspended in a mixture of 0.25 M sucrose – 0.00018 M CaCl_2 . The nuclei were then centrifuged down (International Refrigerated centrifuge head No. 269) for 10 min at 2000 r.p.m. Thereafter, the procedure of HOGBOOM *et al.*⁽¹⁰⁾ was applied. Samples were taken from the whole washed red cells, the stroma-free haemolysate and from the final nucleus preparation. The haemin was extracted as described above.

RESULTS AND DISCUSSION

(a) Experiments with rats

The amount of ^{59}Fe present in one μgm of iron of the nuclear haemoglobin fraction of the liver varied in experiments on rats between 0.6×10^{-4} and 10×10^{-4} % of that injected.

The liver of our rats contained 10 μ gm of nuclear haemin iron out of 2.5 mgm of total iron present in the liver. This fraction makes out 0.4% of its total iron content.

In the course of the purification process in aqueous medium extractable haemoglobin may have been removed. However, the non-extractable haemoglobin content of the nuclei was not found to be larger when the nuclei were isolated after lyophilisation in organic medium. In the haemoglobin content of the nuclear fraction of the red cells of the hen, when

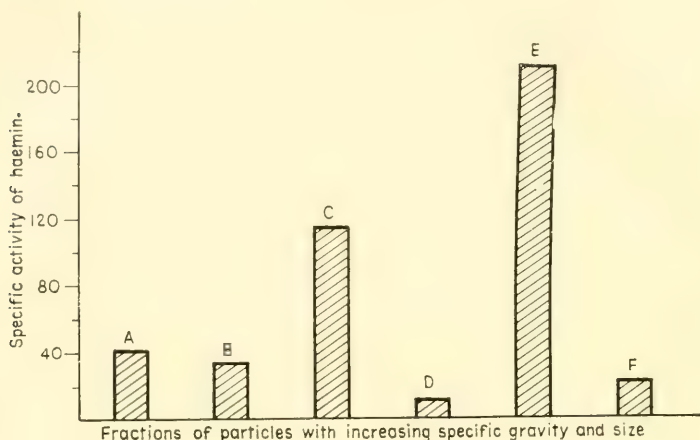


FIG. 1. Results of separation of nuclear components of the liver cells of the rat in LINDAHL's counter-streaming centrifuge.

- A — Total nuclear fraction, prepared according to HOGEBOOM.
- B — Impure mitochondria containing erythrocytes.
- C — Erythrocytes and small fibres of connective tissue.
- D — Cell membranes.
- E — Small nuclei.
- F — Large nuclei.

these were isolated in an organic medium, STERN *et al.*⁽¹⁷⁾ found a haemoglobin-iron content of 19% of that of the total red corpuscle, while we find after isolation of the nuclei in an aqueous medium 1% of the total haemoglobin-iron content to be non-extractable. This 1% compares with 0.4% found by us in the liver nuclei of rats and 2% in those of the rabbit.

We compared also the effect of irradiation with 500 r of X-rays on rats on the incorporation of ^{59}Fe into the liver nuclear haemoglobin fraction. As seen in Table 1 the X-ray effect is shortly after exposure a very restricted one for both haemoglobin fractions. The X-ray effect as seen in Fig. 2 doesn't seem as pronounced as that on the circulating haemoglobin. If we, however, take into account the enhanced activity of the liver of the exposed animal, this difference is strongly reduced.

(b) Experiments with rabbits

The amount of ^{59}Fe present 4 hr after injection in one μgm iron of the non-extractable haemoglobin of the nuclear fraction of the rabbit liver varied between 2×10^{-4} and $6 \times 10^{-4} \%$ of that injected.

The iron content of the non-extractable liver nucleus fraction haemoglobin varied between 0.5 and 3.8 μgm with a mean value of 1.9 μgm per one g liver.

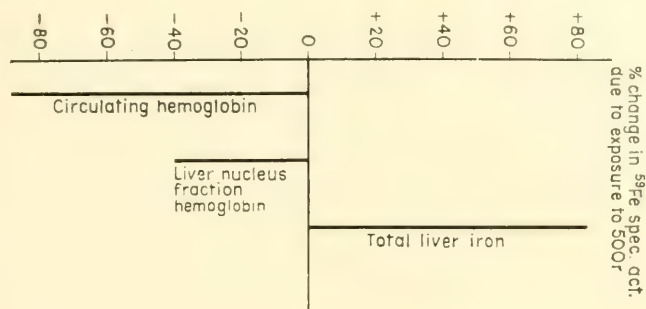


FIG. 2. Effect of exposure of the rat to whole body irradiation on the incorporation of intraperitoneally injected ^{59}Fe as citrate into liver fractions and the circulating haemoglobin.

For the nuclear haemoglobin content of 1 gm of rat and rabbit liver the corresponding figures are 0.38 mgm and 0.56 mgm.

We also carried out experiments with pregnant rabbits 21 days after mating. A plasma sample of the mother was labelled as described by EHRENSTEIN *et al.*⁽¹⁶⁾ The livers of the mothers and foetuses were investigated 11 to 17 hr after labelling the maternal plasma. The results are seen in Table 2.

TABLE 1. — RATIO OF SPECIFIC ACTIVITIES OF IRON FRACTIONS OF TO 500 r EXPOSED AND CONTROL RATS

Injected with ^{59}Fe shortly after exposure			Injected with ^{59}Fe 1 day or more after exposure		
Liver nucleus fraction haemoglobin	Circulating haemoglobin	Total liver iron	Liver nucleus fraction haemoglobin	Circulating haemoglobin	Total liver iron
1.16	0.53	1.60	0.915	0.161	1.15
0.97	1.04	1.41	0.503	0.063	2.48
1.15	0.84	0.93	0.570	0.238	1.72
0.78	0.46	1.27	0.727	0.072	1.92
			0.318	0.029	2.44
Mean value 1.01	0.72	1.30	0.605	0.112	1.94

TABLE 2

	Mother	Foetus
Total liver iron	13.9 mgm	1.5 mgm
Total non-extractable liver nuclear haemoglobin iron ..	24 μ gm	41 μ gm
Total liver activity in % of that injected	8	20
Total non-extractable liver nuclear haemoglobin activity in % of that injected	3.4×10^{-3}	3.4×10^{-1}

Both the iron content of the nuclear fraction and its specific activity is much higher in the foetus than in the mother.

Three of the pregnant rabbits were exposed 1 day prior to injecting them with ^{59}Fe to an X-ray dose of 500 r. The effect of the radiation on the incorporation of ^{59}Fe into the liver nuclear fraction is discussed in a paper by EHRENSTEIN *et al.*⁽¹⁶⁾

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64. APPLICATION OF ISOTOPIC INDICATORS IN HAEMATOLOGY

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Paper read at the Fifth Congress of Haematology

ISOTOPIC indicators have a widespread application in haematology. Their use has broadened our knowledge of the origin and metabolism of the plasma proteins, of the path of porphyrin synthesis and the synthesis of globin and haemin; this application has permitted determination of the life-time of various structural components of the blood, and has facilitated determination of the plasma volume and the amount of circulating red corpuscles, to name only a few regions of application.

In this paper we shall be concerned with the application of radio-iron as an indicator in the study of the metabolism of plasma iron.

THE IRON CONTENT OF BLOOD PLASMA

The diagnostic importance of ascertaining the iron content of blood plasma, the existence of which had already been demonstrated by the experiments of FONTES and THIVOLLE⁽¹⁾, BARKAN⁽²⁾, HENRIQUES and ROCHE⁽³⁾, WARBURG⁽⁴⁾ and WARBURG and KREBS⁽⁵⁾, was early emphasized by HEILMEYER and PLÖTNER⁽⁶⁾. The analytical method worked out by these authors has facilitated the clinical performance of these determinations to a very considerable extent.

After intravenous injection of iron salts, whose iron content, as emphasized earlier by HEILMEYER, should not exceed about 10 mgm, since the ions of iron exert a toxic action, a very rapid migration of part of the injected iron takes place out of the blood fluid while the other, larger, part is responsible for a somewhat more lasting increase of the plasma-iron content. The iron content of the blood fluid, which amounts to about 127 μ gm % in a healthy person, rises after injection of 10 mgm of iron as ferrous chloride to 389 mgm %, but after only 2 hr its value falls to 349 mgm %.⁽⁷⁾ After oral addition of 0.55 gm of iron in the form of ferrous tartrate the iron content of the plasma rises to almost twice the normal value but, after 1 day has elapsed, the normal level of iron is again established⁽⁸⁾. We shall return later to these observations.

THE CAPACITY OF PLASMA PROTEINS FOR COMBINING WITH IRON

The interpretation of the manifold clinical experience that a part of the injected iron disappears very rapidly, as observed earlier by WALDENSTRÖM and others, was made considerably easier by the discovery by HOLMBERG and LAURELL⁽⁹⁾ that the physiological iron of the plasma is attached to a protein fraction in the transferrin. In recent

Gesamt — Eiweiß — 6,35 gm %

Albumin =	41,9 %
α_1 =	9,3 "
α_2 =	13,5 "
β_1 =	14,2 "
γ =	21,1 "
	<hr/> 100,0 %

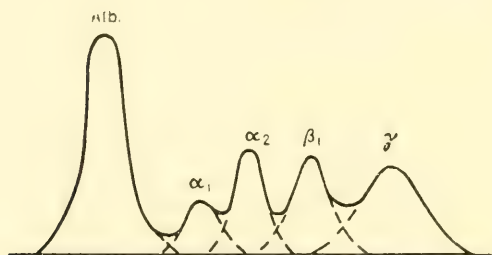


FIG. 1. Localization of the binding of radioactive iron to the β_1 globulin of plasma.

Gesamt-Eiweiß = total protein. Albumin = albumen.

years it has been possible to confirm this result by electrophoretic investigations. For instance, it is evident in Fig. 1, which is taken from the paper of JASINSKI and WUHRMANN,⁽²⁶⁾ that all the labelled plasma iron detected autoradiographically is situated in the same position as the β_1 globulin fraction. The main portion of the transferrin of the β_1 globulin is present in the IV : 7 fraction of the plasma.

If more ferrous sulphate is added to the plasma than can be bound by the amount of transferrin present, then the excess reacts, in contrast to the iron in transferrin, with dipyrindyl. The transferrin of the plasma in a healthy person can combine with about 315 $\mu\text{gm} \%$ of iron.

LAURELL⁽⁸⁾ ascertained the combining capacity of the plasma for iron, both in physiological and pathological conditions in numerous cases, in his comprehensive studies in which he also established the diagnostic importance of this quantity. A plasma iron content of 150 $\mu\text{gm} \%$ may, however, be taken as normal. This applies when it is postulated that the combining capacity of plasma for iron amounts to about 300 $\mu\text{gm} \%$. When the combining capacity for iron is decreased owing to the abnormal synthesis of protein, as in pernicious anaemia, a plasma iron content

of 150 $\mu\text{gm } \%$ is already indicative of a pathological state. Knowledge of the combining capacity of the plasma for iron is therefore of great interest in such cases.

METABOLISM OF PLASMA IRON

The two important quantities, the iron content and the combining capacity of the plasma for iron, can be determined analytically; the metabolic rate of the plasma iron, a quantity no less important than these, cannot be so determined. The ascertainment of the metabolic rate of the iron is made possible by using radioactive iron as an indicator. By labelling the iron of the β_1 globulin and tracing how the radioactivity of the plasma decreases with time, and also how the iron fraction in the organs and in the red corpuscles increases with time, it is possible to trace the path of these iron atoms which were present at the beginning of the experiment in the blood fluid⁽⁹⁾.

At the present time ^{59}Fe is used almost exclusively for labelling iron; it has a half-life of 45.1 days and emits both easily measurable β^- (from 0.26 to 0.46 MeV) and γ -radiation (1.1 to 1.3 MeV).

Labelling of the plasma transferrin is accomplished by incubating a plasma sample in the presence of ^{59}Fe , e.g. as the citrate, at the body temperature for 20 min. If an amount of radioactive iron not exceeding 1 μgm is added to 1 ml of plasma it will be combined quantitatively in

transferrin. If the labelled plasma is now re-injected into the subject, the whole of his transferrin iron in the plasma will thereby be labelled. In animal experiments iron may also be added to a donor, possibly even in larger quantities and, e.g. labelled plasma may then be transferred from the donor to the acceptor after 1 hr has elapsed.

FLEXNER and his co-workers⁽¹¹⁾ were the first to label the plasma transferrin of guinea-pigs by the injection of radioactive iron and to determine the rate at which the iron escapes from the blood fluid. Labelling the transferrin iron by injecting iron into the subject is frequently inapplicable

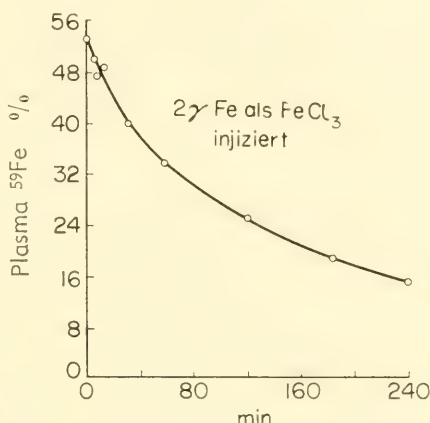


FIG. 2. Loss of labelled iron from rabbit plasma after intravenous injection of 2 μgm of iron. 2 μgm Fe injected as FeCl_3 (caption on the graph).

cable since a considerable part of the injected iron is transported into the organs, before it has the opportunity to be incorporated in the transferrin, and may complicate the experimental results by its presence in them. Even when the very small amount of $2\text{ }\mu\text{gm}$ of iron is injected

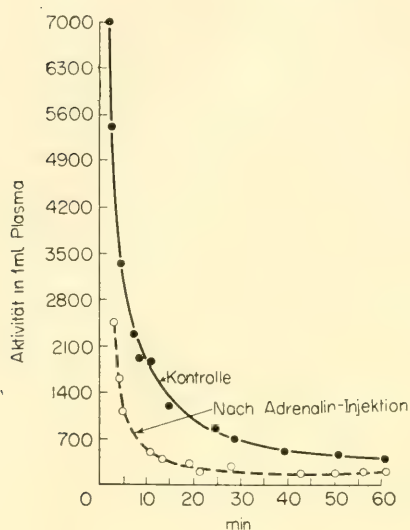


FIG. 3. The effect of adrenalin on the rate of escape of labelled phosphate from the plasma.

Kontrolle = control test;
 Nach Adrenalin-Injektion
 = after injecting adrenalin;
 Aktivität in 1 ml Plasma
 = activity per ml plasma.

as citrate or chloride into a rabbit, i.e. one-hundredth of the amount which the still available transferrin can combine with, this iron does not combine completely with the plasma transferrin, but a part of it leaves the plasma before having an opportunity to combine with the protein. It is evident in Fig. 2, which is taken from a study performed in our laboratory by GIULIANO, that this process is concluded after a period of only 4–5 min and that ^{59}Fe still present then disappears from the plasma of the rabbit with a half-life of about 2 hr, which is characteristic of the metabolism of physiological plasma iron.

VAHLQUIST and co-workers have already observed the very rapid disappearance of 0.5 to 2.0 mgm iron, after injection into a rabbit, from the plasma⁽²⁸⁾, but the radioactive method makes it possible also to follow the course of very small amounts of iron, $1\text{ }\mu\text{gm}$ or less,

and to measure both the unilateral loss of iron and the renewal rate of plasma iron.

By making use of the ready accessibility of the radioisotopes of sodium, potassium, etc. it was shown at an early date that the rate of replacement of these ions may be extraordinarily high⁽¹²⁾. In the course of 2 min about half of the sodium ions present at the beginning of the experiment in the plasma of a rabbit is replaced by extravascular sodium ions. A rapid disappearance of injected iron salts such as that indicated in Fig. 2 is therefore not very surprising. The iron combined in the transferrin, on the contrary, escapes relatively slowly with a half-life of from 70 to 120 min from the human circulatory system and still more slowly from the rabbit. Experiments with transferrin labelled with ^{131}I showed that this compound leaves the blood fluid with a half-life of a few days⁽¹³⁾. Iron to a very large extent escapes from the plasma not in a form com-

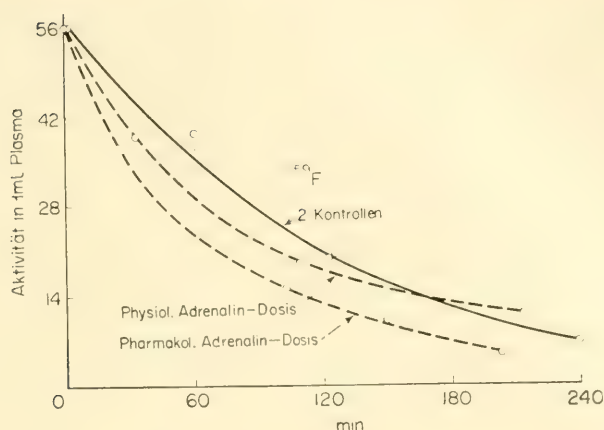


FIG. 4. The effect of adrenalin on the rate of escape of iron combined in β_1 globulin from the plasma.

Kontrolle = control test. Physiol. Adrenalin-Dosis = physiological dosage of adrenalin. Pharmakol. Adrenalin-Dosis = pharmacological dosage of adrenalin. Aktivität in 1 ml plasma = activity per ml plasma.

bined with protein but only after it has been split off from the transferrin, and it is very probable that the process determining the rate of escape is the dissociation of iron transferrin since the iron ions or iron-containing radicals which are split off penetrate the capillary walls with the greatest ease.

Plasma constituents such as phosphate, for instance, which transfer extraordinarily quickly into the extracellular space, often return before they "finally" leave the plasma. This state is the more quickly established when the phosphate has the opportunity of finding an additional way of entering into the cell. The reversion of phosphate ions into the plasma is thereby made more difficult and its ultimate disappearance from the blood fluid is thus facilitated. If the disappearance of phosphate from the plasma is accelerated, e.g. by the injection of adrenalin, the effect must be ascribed in a high degree to an accelerated entry of the phosphate from the extracellular space

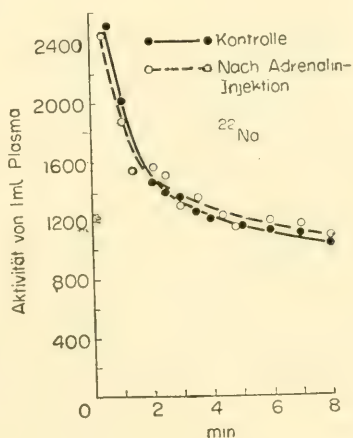


FIG. 5. The effect of adrenalin on the rate of escape of labelled sodium from the plasma.

Kontrolle = control test.

Nach Adrenalin-Injektion = after injecting adrenalin. Aktivität von 1 ml plasma = activity per ml plasma.

into the tissue cells, owing to an enhanced metabolism taking place in the cells at this time. Figures 3 and 4 illustrate this type of accelerating effect of adrenalin on the escape of labelled phosphate and labelled iron from the plasma. The rate of escape of sodium ions (Fig. 5), on the contrary, is not affected by the injection of adrenalin. Sodium is essentially an extracellular element, although it is incorporated in not inconsiderable amounts into the skeleton and also into the cells but this process of incorporation is not appreciable during the short experimental time of close to 10 min. In this instance, the exchange process takes place almost solely between the plasma and the extracellular space and its rate is therefore not accelerated by the injection of adrenalin⁽¹⁹⁾.

The investigation by FLEXNER and co-workers on the rate of escape of labelled iron from the blood plasma was followed by numerous other studies by LAWRENCE and co-workers⁽¹⁵⁾ and by many other workers. HUFF and associates calculate the daily metabolism of plasma iron from the formula⁽¹⁶⁾:

Iron metabolism (mgm/day) =

$$\frac{0.693 \times 24 \text{ hr /day} \times \text{Fe mgm/ml} \times \text{plasma volume (ml)}}{\text{half-life of the disappearing } ^{58}\text{Fe (hr)}}$$

They find that the daily plasma-iron metabolism per kgm of body weight in a healthy person is equal to 0.4—0.45 mgm, whereas it is as low as 0.205 mgm in pathological case such as a refractory anaemia and as high as 3.93 mgm in a case of haemolytic anaemia.

By determining a fraction of the plasma-⁵⁹Fe which has accumulated after a definite time in the various organ fractions and in the red blood corpuscles, an explanation is obtained of, among other things, that rather more than one-half of the metabolized iron in a healthy person is used for the synthesis of red blood corpuscles and that this value can fall to one-eighth in refractory anaemia. The metabolism of iron in the red blood corpuscles was calculated from the formula⁽¹⁵⁾:

$$\frac{^{59}\text{Fe in the red blood corpuscles}}{\text{initial value of } ^{59}\text{Fe content in the plasma}} \times \text{iron metabolism in the plasma}$$

THE CONVEYANCE OF IRON FROM THE ORGANS INTO THE PLASMA

The combining capacity of transferrin for iron is only about one-half utilized in healthy people and also in rabbits, and at first glance it appears remarkable that if indeed a better utilization of this combining capacity can be achieved by parenteral or oral administration the

enhancement attained is only temporary. A more permanent increase of the iron content in the plasma is spoiled by the fact that an increase of the iron level is accompanied by an increased rate of escape of iron from the plasma and, therefore, an increased rate of entry of iron from the organs is required to maintain the increased level of iron. By the oral administration of iron tartrate LAURELL⁽⁸⁾ attained almost double the iron content in the plasma of a healthy person, but the normal plasma-iron level was re-established after 20 hr. After the injection of 10 mgm of iron, TOTTERMAN⁽⁷⁾ found the 2 hr value to be only about 15 per cent less than the 5 min value, indicating a partial compensation of the escaping plasma iron by the iron from the organs.

We found the daily metabolism of plasma iron⁽¹⁷⁾ in a rabbit weighing 2.6 kgm to be about 800 μ gm; of this somewhat more than 400 μ gm is used to replace the decayed blood corpuscles while only 80 μ gm is taken up by the liver. The 400 μ gm of iron which is used daily for forming haemoglobin appears again after the decay of the blood corpuscles which contain this compound. Only small amounts of iron are absorbed daily by the digestive organs.

The daily uptake of iron of the liver, which contains 7 mgm iron, from the plasma amounts to 80 μ gm and this must be compensated by a corresponding release to the plasma since, otherwise, the iron content of the liver would be doubled in the course of 3 months. The liver takes up these small quantities only from the transferrin of the plasma; in a period of 6 hr the liver absorbs almost one-half of 0.5 mgm of iron salt injected into a rabbit. Of 1.6 gm of "ferrivenin" injected, 84 per cent was ofund in the liver of an infected patient⁽²⁴⁾.

The liver of a rabbit which had been injected for several months with iron (viviferrin) and which had an iron content of 35 mgm absorbed only 40 μ gm daily from the plasma⁽¹⁷⁾. The iron turnover in the plasma of such rabbits is greater than in normal animals. A 30 per cent higher content of haemoglobin, 12.9 gm % instead of 9.8 gm %, with an almost unchanged content of plasma iron, 151 μ gm % instead of 145 μ gm %, was essentially responsible for the increased metabolism from 800 to 920 μ gm %.

It is not likely that a liver burdened with iron will release less iron than a normal one, and since it absorbs less from the plasma than does the latter, some latitude becomes available for the release of its iron burden. An investigation by ANDERSON⁽¹⁸⁾ indicates that this does occur. While 18.4 mgm % iron was present 3 months after injecting iron into a liver burdened with iron, the content decreased to 16.1 mgm % after 6 months.

Generally the liver is regarded as the main organ for storing iron, and the ferritin of this organ as the compound which yields iron to the plasma. MAZUR and his co-workers⁽¹⁹⁾ have recently produced a proof that a small portion of the ferritin iron is present on the surface of the

ferritin molecule as a mixture of ferrous and ferric compounds. The equilibrium between the ferric-disulphide—ferritin and the ferrosulphydryl—ferritin is displaced in favour of the ferro-form (see also BIELIG and BAEYER⁽²⁷⁾) by venesection, which leads to anoxia, or by reducing agents such as glutathione, and the ferrous iron is conveyed into the plasma for formation of iron transferrin.

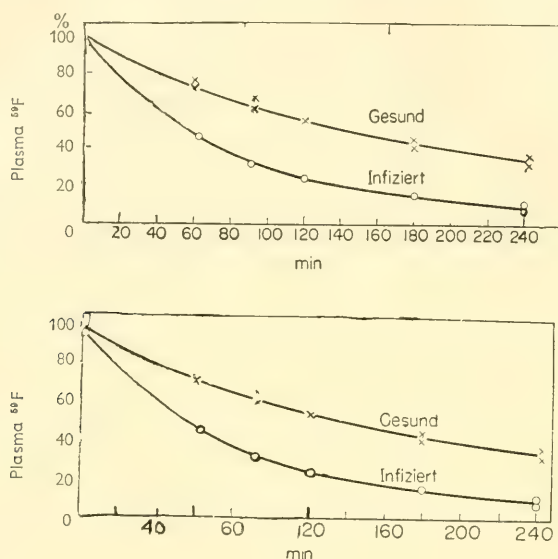


FIG. 6. Rate of escape of iron combined in β_1 globulin from the plasma of normal and infected rabbits.
Gesund = healthy. Infiziert = infected.

It is highly probable that the lack of iron in the plasma of infectious diseases is partly attributable to the disturbed transference of the ferritin iron into the plasma.

In infectious diseases, of course, not only is there inhibition of the entry of iron into the plasma but there is also acceleration of the escape of iron from the plasma into the organs in which a more rapid metabolism now occurs. This is illustrated in Fig. 6, which presents experimental results obtained in our laboratory by EHRENSTEIN⁽²⁰⁾, who has compared the rate of escape of iron globulin, labelled with iron, from the plasma of normal rabbits and rabbits infected with *Pasteurella multocida*. As has already been mentioned, an accelerated uptake of iron by the cells of the storage organs leads to an accelerated escape of iron ions from the plasma. Since, however, the level of iron in the infected plasma amounted only to about half of the normal, the amount of iron escaping from that plasma in unit time was not very much larger than that released from the plasma of normal animals.

The possibility must also be considered that conditions exist in infectious diseases which promote dissociation of the iron transferrin, a step which must precede the penetration of the protein-linked iron through the capillary wall, and thus these conditions accelerate the escape of iron from the blood fluid.

The escape of iron from the plasma and the entry of iron into it are two independent processes which are presumably coupled by means of hormones. Since the daily metabolism of iron amounts to about 30 mgm and the uptake from the gut constitutes only a small fraction of this quantity, the metabolism takes place essentially between the plasma, the intercellular fluid, the organs and the red blood corpuscles. The rate of escape of iron from the plasma is promoted by an increasing haemopoiesis and also by an enhancement of the metabolism taking place in the storage organs. The two processes are possibly responsible for the 20 to 90 per cent increase of plasma-iron level observed in the early hours of the day.

In animals which have been deprived of the suprarenal capsule, and still more powerfully in normal dogs, adrenalin leads to a temporary lowering of the iron level, whereas ACTH causes this effect only in normal animals. CARTWRIGHT and co-workers²¹ even found that an intramuscular injection of physiological saline solution lowers the plasma iron content in dogs by from 0 to 41 per cent, which presumably also is brought about by a hormonal effect. Oestrogen raises and androgen lowers the amount of iron stored in the liver of chickens⁽²²⁾ and, therefore, these hormones also should affect the escape of iron from the plasma or its entry from the liver, or both of these processes.

As LAURELL^{23, 25} has already found, it is highly probable that iron dissociated from the protein compound, and not iron transferrin, which is transferred from the plasma into the extravascular extracellular space. With the aid of paper-electrophoretic methods, EHRENSTEIN of our laboratory has recently proved that the iron present in the lymph, and therefore the iron present in the spaces between the cells, is also combined with β_1 globulin. He found that the iron content of the lymph of the rabbit is one-half to one-third the content in the plasma, which amounted to about 150 mgm %. He also found that the combining capacity of the lymph for iron, and therefore the transferrin content, is also about one-third that of the plasma. LAURELL⁽²³⁾ pointed out that an importance attaches to the ratio $(\text{Fe transferrin content})/(\text{Fe-free transferrin}) = K[\text{Fe}^{+++}]$ in respect of the metabolism of iron, and it is not without interest that this ratio is found to be about the same in the plasma as in the lymph. Since the iron content of the intercellular space should be lower than that of the lymph, the values quoted represent an upper limit for the intercellular space and a lower limit for the space within the cells. The volume of the intercellular space has been assumed to be four times the plasma volume.

Figure 7 shows the transport of iron from the labelled transferrin iron of the plasma of a normal rabbit into the intercellular and cellular space. The iron disappearing from the plasma which is not found in the intercellular space has already been transferred into the cells.

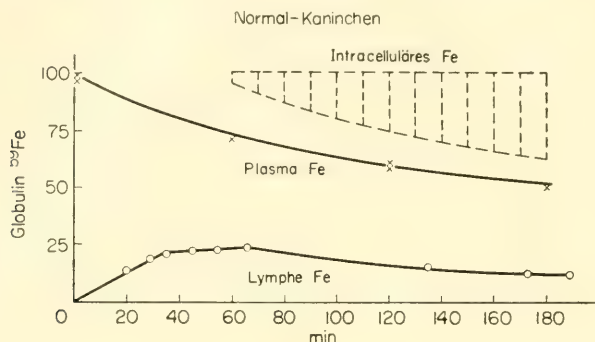


FIG. 7. Transport of iron from the labelled globulin iron of the plasma of a normal rabbit into the intercellular and cellular space.

Normal Kaninchen = normal rabbit.

Intracelluläres Fe = intracellular iron.

Plasma Fe = plasma iron.

Lymphe Fe = lymph iron.

Figure 8 shows the powerfully accelerated transport of globulin iron from the plasma of an infected rabbit; this acceleration is partly attributable to the fact that the turnover of red corpuscles (shorter life-time) is accelerated in the infected animal.

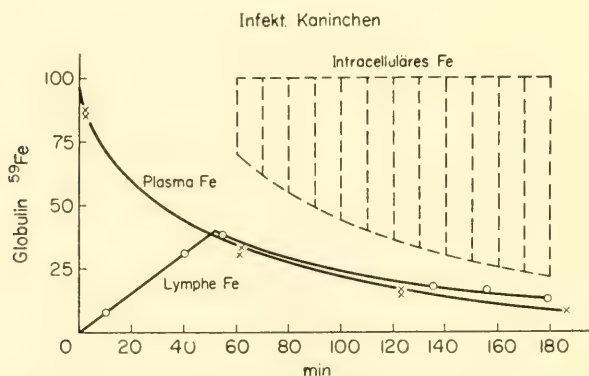


FIG. 8. Transport of iron from the labelled globulin iron of the plasma of an infected rabbit.

Infekt. Kaninchen = infected rabbit.

Intracelluläres Fe = intracellular iron.

Plasma Fe = plasma iron.

Lymphe Fe = lymph iron.

In contrast to the globulin iron, the non-physiologically bound iron has been shown by electrophoretic studies to pass into the intercellular space essentially free from transferrin, as shown in Fig. 9; this diagram shows the distribution of non-physiologically bound iron introduced into the plasma, in the intercellular space and the cellular space of a normal rabbit.

The problems mentioned in the introduction can be solved in other, though more tedious ways, e. g. by applying isotopic indicators, but a determination of the iron turnover cannot be considerate without the

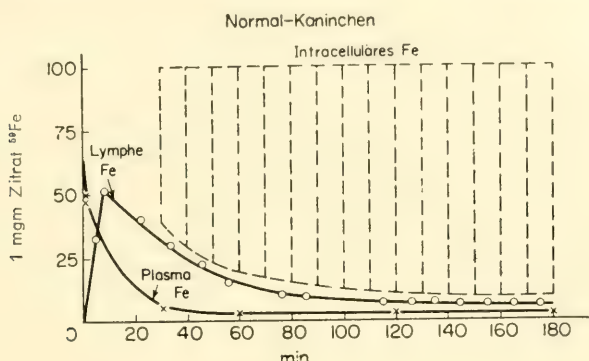


FIG. 9. Distribution of non-physiologically bound iron (1 mgm) added to the plasma among the plasma, the intracellular space and the cellular space of a normal rabbit.

Normal-Kaninchen = normal rabbit.

Intracelluläres Fe = intracellular iron.

Lymph Fe = lymph iron.

Plasma Fe = plasma iron.

1 mgm citrate ^{59}Fe added as citrate.

use of radioactive tracers. Use of these indicators provides a possibility of splitting up into components the dynamic equilibrium whose resultant is the plasma iron level. The result of splitting up this equilibrium directs our attention, with particular emphasis, to the importance of the deficient flow of iron from the storage organs into the plasma in infectious diseases and to some other pathological cases. In order to attain a permanent increase in the plasma iron level in such diseases it would be necessary to increase the deficient flow into the plasma.

That the lymph flow plays a part in maintaining a normal plasma iron level is shown by the observation of EHRENSTEIN, viz. that the plasma iron level in a rabbit falls on the average from 191 to 146 $\mu\text{gm} \%$ 4 hr after removal of the ductus thoracicus and the truncus jugularis sinister, whereas the level remains constant in control rabbits which have been subjected to operation without removing the ductus thoracicus.

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65. NOTE ON THE DETERMINATION OF RADIOIRON

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SHORTLY after radioiron became available, HAHN, BALE, LAWRENCE and WHIPPLE (1939) applied this radioisotope with much success to the study of the absorption and distribution of iron in the animal body. An analytical method was devised by HAHN, BALE and BALFOUR (1942) which permits the determination of the iron content of a tissue fraction and its radioactivity. Following wet ashing of the tissue and after addition of a known amount of carrier the solution is electrolysed and the iron deposited on a copper planchet which is then placed under the Geiger counter. The yield of electroplating is tested by dissolving the electroplated iron in acid and carrying out a colorimetric determination of the solution.

TABLE 1. — ADDED AND RECOVERED
RADIOIRON ^{59}Fe AS FERRICHLORIDE WAS
PIPETTED INTO TEST TUBES, 500 MICRO-
GRAM OF CARRIER IRON ADDED, AND
THE PROCEDURE DESCRIBED IN THE
TEXT CARRIED OUT

^{59}Fe added (counts per min.)	^{59}Fe recovered (counts per min.)
10	10.2
20	19.7
30	29.2
100	98.0
200	200.0
300	294.0

Though this analytical method proved to be of great importance in radioiron studies, we were induced to replace it by a much more rapid, simple, and certainly not less exact one since a long series of radioiron determinations in numerous tissue fractions was to be carried out.

The new procedure is based on (a) colorimetric determination of the iron content of a solution of a tissue fraction after wet ashing, (b) addition

of carrier iron as FeCl_2 to bring up the iron content of the sample to a total amount of 500 microgram, (c) precipitation of the solution with H_2S after neutralizing it with ammonia, (d) collection of the FeS obtained on a filter paper placed on the bottom of a perforated aluminum dish of 1.2 cm diameter and 2 mm depth (as used in the determination of the radioactivity of ammonium magnesium phosphate precipitates), (e) plac-

TABLE 2. — SERUM FROM HUMAN SUBJECTS PREVIOUSLY INJECTED WITH SERUM CONTAINING ^{59}Fe 1 ML SERUM WAS DRIED AT 100°C , WET ASHED WITH 1 ML SULFURIC ACID AND SOME PERHYDROL AND ITS ACTIVITY DETERMINED AS DESCRIBED

Sample	Relative active units per ml of serum		
	Combustion 1	Combustion 2	Combustion 2
1	269	230	234
2	194	171	—
3	137	133	141
4	108	98	100
5	79	77	79
6	62	62	63
7	1.9	1.9	3.3
8	2.8	2.0	2.5
9	263	267	275
10	35	35	37
11	19	20	23
12	6.3	5.5	6.8
13	10	10	10

TABLE 3. — DETERMINATION OF ^{59}Fe CONTENT OF HEMIN ISOLATED FROM HUMAN ERYTHROCYTES. COMBUSTION AS STATED IN TABLE 2, IRON DETERMINED COLORIMETRICALLY WITH SULFOSALICYLIC ACID

Sample	Activity units per microgram iron	
	Combustion 1	Combustion 2
1	0.37	0.35
2	0.24	0.24
3	0.16	0.16
4	0.031	0.029
5	0.049	0.051
6	0.27	0.23
7	0.24	0.23
8	0.27	0.29

ing the aluminum dish under the Geiger counter. Since the 500 microgram of iron are about equally distributed over a surface of 1.2 cm² we are measuring the radioactivity of an almost infinitely thin iron layer. The intensity of the β -radiation emitted by ⁵⁹Fe is reduced to half its value when passing a layer of 10.0 mgm of aluminum per cm².

A few examples of the method described are given in Table I which contains data on added and recovered amounts of ⁵⁹Fe obtained when preparing "standard" samples.

Since the procedure is rapid and easy to carry out and requires no special equipment but a Geiger counter, it can be used in most clinical laboratories. The accuracy obtained in clinical application of the method is seen from Tables 2 and 3. Duplicate determinations involving both combustion and counting technique are stated.

Summary

A simple and rapid method for the determination of ⁵⁹Fe is described. The radioiron is precipitated as FeS and after filtration of the solution counted on the filter paper.

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COMMENT ON PAPERS 61-65

THE method of *in vitro* labelling of plasma by adding minute amounts of iron of high specific activity worked out in the Donner Laboratory by HUFF *et al.* (1950) proved to be most useful and found a very extended application. In paper 61 by making use of this device, the effect of adrenalin on the rate of extrusion of ^{59}Fe from the plasma was investigated. The marked increase of extrusion rate observed can hardly be due to the effect of adrenalin on the permeability of the capillary wall, since extrusion of ^{22}Na from the plasma was not found to be accelerated after injection of adrenalin. It is due to an increased rate of uptake of ^{59}Fe by the tissue cells.

Even when exposing a rabbit to a heavy dose of 1500 r, 1 hr after exposure the rate of extrusion of ^{59}Fe from the plasma, which is mainly due to a transport to the bone marrow, is not much reduced. If we wait for 48 hr after exposure, the effect of 800 r is found to be more effective in reducing the exodus of ^{59}Fe from the plasma than are 1500 r after the lapse of 1 hr. HENNESSY and HUFF (1950) have already previously shown that the optimal depression of ^{59}Fe incorporation into erythrocytes is obtained 1 to 2 days after exposure. This result, and the observations stated in paper 61 suggested the explanation that it is not the haemoglobin synthesis which is radiosensitive, but the blocking of haemoglobin formation under the effect of irradiation is due to interference with cell division and to cell destruction. The correctness of this view was brought out by the autographic investigations of LAJTHA *et al.* (1955). In following up cellular processes the autographic technique proved to be a most powerful line of approach.

As described in paper 62 the nuclear fraction of the rat and rabbit liver contains a small amount of haemoglobin non-extractable in aqueous medium. The incorporation of ^{59}Fe into this fraction was also found to be radiosensitive, not however its incorporation into ferritin or cytochrome b. Due to the blocking of incorporation of ^{59}Fe into haemoglobin under the effect of irradiation, the specific activity of ferritin, ferrosiderin and other iron compounds of the liver increases. Quite recently THEORELL and ÅKESON succeeded in preparing highly pure myoglobin. By availing ourselves of this method when isolating myoglobin from rat muscles we found the hemin moiety of myoglobin to be radiosensitive only.

Since the availability of the scintillation counter the activity of blood and other labelled iron containing samples is mostly measured by making use of this apparatus. Prior to the availability of the latter we precipitated the iron after wet combustion of the sample as sulphide and measured the activity of the precipitate with the Geiger counter as described in paper 65. Minute activities are still preferably measured by making use of the last-mentioned procedure.

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66. EMBRYONAL IRON TURNOVER.

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VOSBURGH and FLEXNER (1950) were the first to inject labelled iron as FeCl_3 into the circulation of the guinea-pig and to study the passage of radioiron through the placenta. The amount of iron passed into the foetus of the guinea-pig varied between 16 and 119, with an average of 56 microgram of iron per gram of placenta per day. They found no correlation with gestation age. This finding induced them to suggest that the passage of iron across the placenta appears to involve a different mechanism than that which is concerned with other substances which they studied. The rate at which water, sodium and inorganic phosphate cross a unit weight of the guinea-pig's placenta increases about 10 times during the last half of pregnancy. This increase of rate is correlated with thinning of the barrier between maternal and foetal circulation and increased area of exchange, and is in the predicted direction if the process is essentially diffusion. Iron, however, crosses the placenta at a rate which shows no correlation with the duration of pregnancy, i. e. there is no evident difference between the rates in early and late stages. In addition, there may be a considerable difference in the amount of iron transferred to members of the same litter during the course of the experiment. They emphasize that these characteristics of iron transport across the placenta suggest the existence of a rather complex regulatory mechanism which may be analogous to that concerned with the absorption of iron from the gastrointestinal tract, since ferritin has been demonstrated in the placenta of the guinea-pig by LATHAM and VOSBURGH (1950) and in the human placenta by MAZUR *et al.* (1955). The above view recently received much support by the work of WÖHLER (1955), discussed by him and by HEILMEYER (1956). WÖHLER injected 1.25 mgm of labelled ferrosulfite into the circulation of pregnant rabbits, and found the ferritin extracted from the placenta to show a marked radioactivity already 40 minutes after injection. He demonstrated, furthermore, the marked dependency of the total iron content of the placenta and the foetal plasma iron on the plasma iron content of the mother, in contrast to the ferritin content of the placenta. The latter

varied to a restricted degree only with a varying maternal plasma iron level. From these observations he inferred that much of the maternal plasma iron reaches the embryo after being previously incorporated into ferritin of the placenta.

We wished to study iron metabolism at an early stage of pregnancy in which the liver of the embryo is to a large extent involved in hemopoiesis and furthermore, the effect of exposure to radiation on iron turnover in the pregnant rabbit.

Plasma samples of control and irradiated rabbits obtained 21 days after mating, were incubated at 37° C for 20 minutes with 0.5 microgram of iron as citrate labelled with ^{59}Fe of 5 microcurie of activity per one ml plasma, and then reinjected. The animals were killed 11.5 to 17 hours after injection. The activity and iron content of the plasma of the mother, total iron and activity of the livers of the mother, and the foetus and the specific activity of their liver-ferritin was then determined. We measured also the specific activity of the circulating hemoglobin and of the hemoglobin present in the nuclear fraction of the liver of mother and foetus. The amount of radioiron found in the embryos after removal of the liver was determined as well. The weight of the rabbits varied between 3.2 and 4.1 kgm the number of embryos between 4 and 11, their aggregate weight between 11 and 58 gm, and the aggregate liver weight of the embryos between 1.4 and 5 gm.

After wet ashing of the samples to be investigated, a known aliquot of the sample was used for colorimetric determination of its iron content. The iron determinations were made essentially according to the sulfosalicylic acid method by LORBER (1927). Another known aliquot of the ashed sample, after bringing its iron content up to 500 microgram by adding FeSO_4 , was precipitated as sulphide and filtrated through perforated aluminium dishes covered with filter-paper, as described previously by AGNER *et al.* (1954), prior to placing the dish under the Geiger counter.

The livers of the animals were homogenized in 9 vol. 0.25 molar sucrose containing 0.0018 M CaCl_2 , centrifuged for 10 min at 2000 r. p. m. (Internat. refrigerated centrifuge horizontal head No. 269), and the ferritin was precipitated from the supernatant by addition of half a volume of ammoniumsulphate. The precipitate was taken up in water and repeatedly fractionated with ammoniumsulphate. The precipitate was then dissolved in water, heated to 70° C for a few minutes and the cold solution filtered. The filtrate, which contained the ferritin, was then analysed.

The hemin of the purified red corpuscles was extracted with a mixture of acetone and HCl (10 ml 20 p. c. HCl in 1 liter of acetone). It was twice recrystallized from acetic acid and the crystals were washed with diluted HCl. The non-water soluble hemoglobin of the liver fraction was obtained from thoroughly purified nuclei, prepared according to Hogeboom-Schneider as described by BONNICHSEN, EHRENSTEIN and HEVESY (1956).

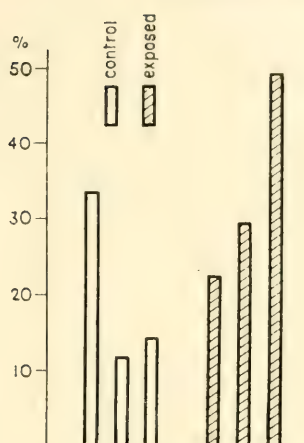


FIG. 1. Percentage of ^{59}Fe injected into the mother present in the embryo.

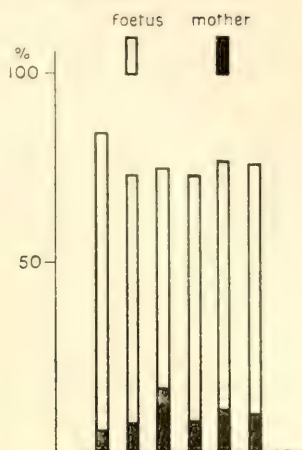


FIG. 2. Percentage of embryonal resp. maternal ^{59}Fe present in the liver.

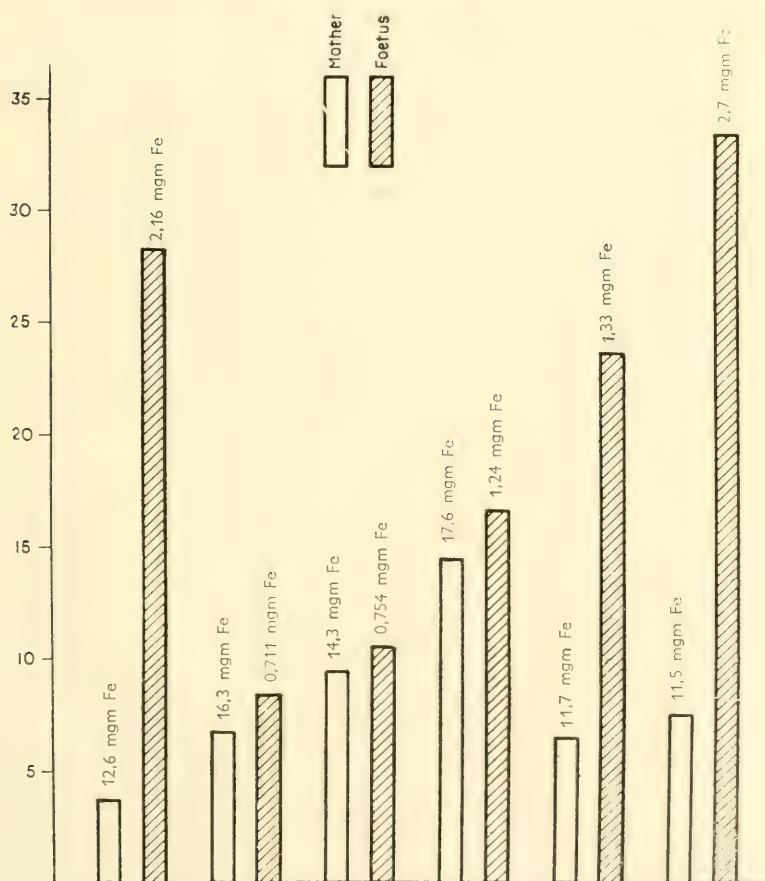


FIG. 3. Percentage injected ^{59}Fe and total iron present in the maternal and foetal liver.

RESULTS

It has been shown repeatedly (HENNESSY and HUFF 1950, HUFF, BETHARD, GARCIA, ROBERTS, JACOBSEN and LAWRENCE, 1950; BELCHER, GILBERT and LAMERTON, 1954; BONNICHSEN and HEVESY, 1955 (*a*)) that about one day or later after exposure of the animal to

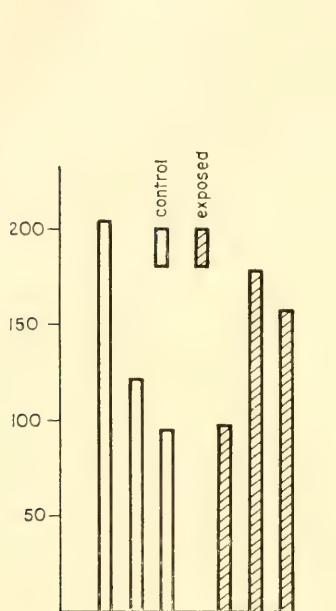


FIG. 4. Ratio of ^{59}Fe uptake by embryonal and maternal liver of equal weight.

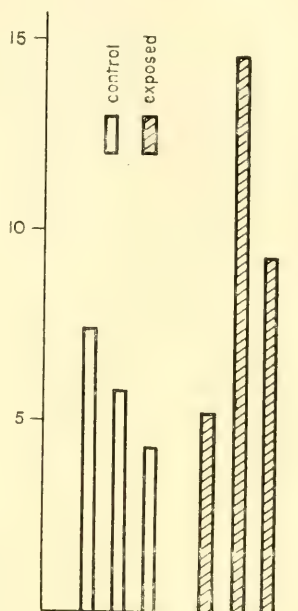


FIG. 5. Ratio of the specific activities of embryonal and maternal circulating hemoglobin.

radiation the incorporation of ^{59}Fe into hemoglobin being strongly depressed, more ^{59}Fe makes its way into the liver and other non-hemopoietic organs than in non-exposed controls. To the same reason is due at least partly, the enhanced passage of the mother-injected ^{59}Fe into the embryo of exposed rabbits, as seen in Fig. 1. A comparison of the ^{59}Fe uptake by the liver of the mother and the embryo in control and exposed rabbits is made difficult by the varying number of embryos and thus of the embryonal livers and their weight in the different experiments. The conspicuous concentration of ^{59}Fe in the embryonal liver is however clearly demonstrated by Fig. 2. This in spite of the much lesser weight of the embryonal liver (2, 5, 2, 1.4, 1.5, 2.0, 2.0 and 3.9 gm) than that of the maternal liver (86, 138, 128, 170, 95 and 100 gm). The uptake of ^{59}Fe by the maternal and foetal liver and their iron content

is compared in Fig. 3, while Fig. 4 demonstrates the ratio of ^{59}Fe uptake by 1 gm of foetal and 1 gm of maternal liver, this ratio reaching a maximum figure of 204 and having a mean value of 143.

The ratio of the mean specific activity of the maternal liver ferritin iron and total liver iron was found to be 1.2, that of the corresponding figure for the embryo 1.0. In view of the fact that the blood content of the liver cannot be fully removed, no conclusion can be drawn from the above difference.

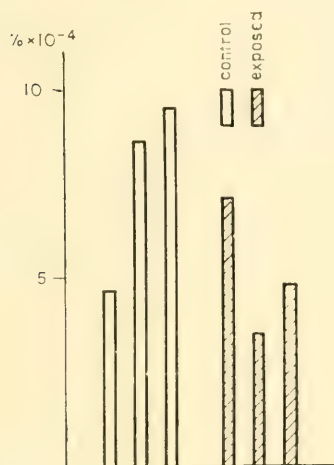


FIG. 6. Percentage of the activity of 1 μgm maternal hemoglobin in the total maternal activity at the end of the experiment $\times 10^4$.

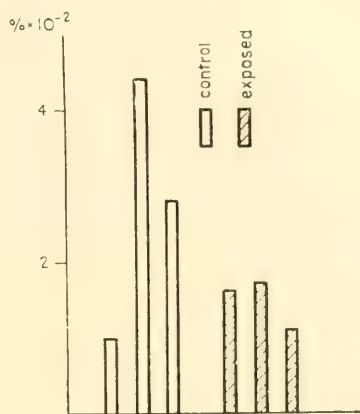


FIG. 7. Percentage of the activity of 1 μgm embryonal circulating hemoglobin in the total embryonal activity at the end of the experiment $\times 10^2$.

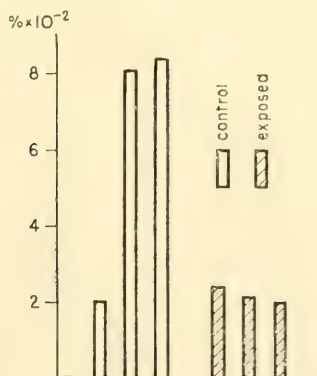


FIG. 8. The activity of 1 μgm embryonal nuclear hemoglobin Fe in per cent of the total embryonal activity 10^2 .

CIRCULATING HEMOGLOBIN

For the above-mentioned reason the ratio of the specific activities of the hemoglobin iron of the embryo and the mother, as seen in Fig. 5 is larger in the exposed animal than in the control rabbit (mean ratio = 1.66). When comparing the percentage of activity present in the maternal organism at the end of experiment with the activity of $1 \mu\text{gm}$ hemoglobin iron, the data seen in Fig. 6 are obtained, while Fig. 7 shows the corresponding figures for the embryonal organism. Both figures indicate depressed hemopoiesis.

LIVER NUCLEAR HEMOGLOBIN

As found by BONNICHSEN and HEVESY (1955 *b*) the nuclear fraction of the liver contains small amounts of hemoglobin inextractable with aqueous solution. The depressed hemopoiesis of this hemoglobin fraction

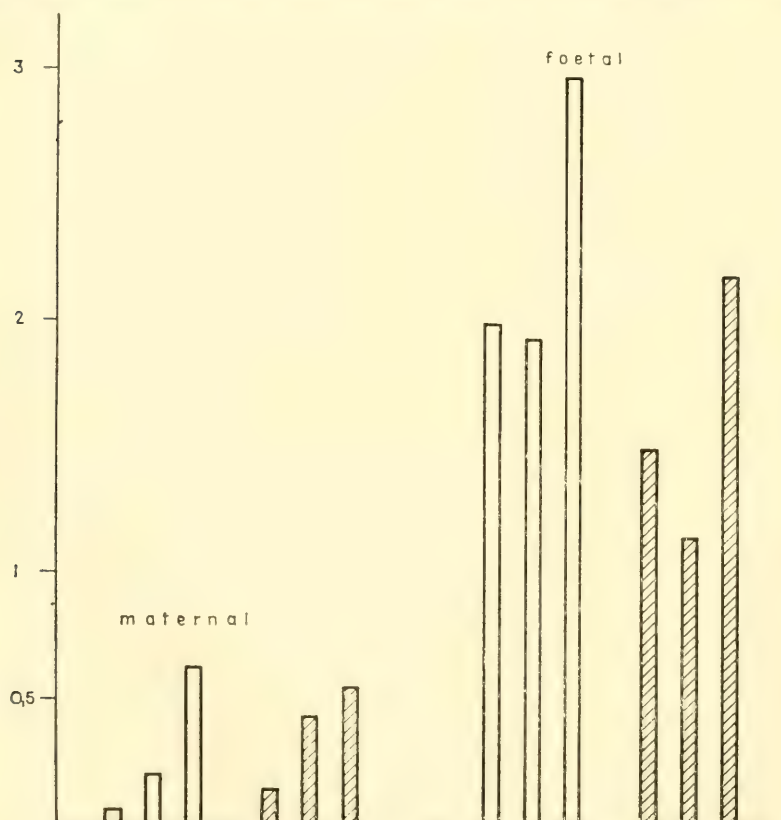


FIG. 9. Ratio of the specific activity of nuclear hemoglobin Fe and circulating hemoglobin Fe.

in the embryo of the exposed rabbit is seen in Fig. 8. The specific activity of the iron of this fraction is much smaller than that of the iron of circulating hemoglobin in the maternal organism, but appreciably larger in the embryo (cf. Fig. 9).

Presumably hemoglobin synthesis takes place besides in the cytoplasm of the hemopoietic liver cells in their nuclei as well. It is of interest in this connection to recall the finding of STERN, ALLFREY, MIRSKY and SAETREN (1952), according to which almost $\frac{1}{5}$ of the hemoglobin of the avian nucleated red corpuscles is present in the nuclei.

In our experiments during the purification process of the nuclei, the water soluble hemoglobin, present possibly in appreciable amounts, may have been removed, in contrast to the small amounts of water insoluble hemoglobin which amounted to not more than 4.1 mgm in our maternal and 17.5 mgm in our foetal samples. It was found in a previous investigation [BONNICHSEN, (1956)] that the radiation sensitivity of the nuclear hemoglobin becomes very marked after the lapse of about 1 day, as does that of the circulating hemoglobin.

Summary

Seventeen hours after labelling the circulating iron- β_1 globulin with ^{59}Fe , 14–34 per cent of the labelled iron is found in the 21 days old foetus of the rabbit.

Exposure of the rabbit to 500 r of X-rays increases the amount of ^{59}Fe passing the placenta to almost twice the value in non-exposed animals.

Seventy-three–eighty-three per cent of the ^{59}Fe content of the embryo is found in the liver.

The specific activity of the hemoglobin iron present in the nuclear fraction of the liver amounts to one fourth of that of the circulating hemoglobin in the maternal and to about twice in the embryonal organism.

Exposure to radiation one day prior to labelling the plasma depresses the fraction of embryonal ^{59}Fe incorporated into the hemoglobin of the nuclear liver fraction to about half of the value observed in non-irradiated rabbits.

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COMMENT ON PAPER 66

As already shown by VOSBURGH and FLEXNER (1950) the ^{59}Fe introduced into the maternal plasma of pregnant guinea-pigs is soon detectable in the embryos. In paper 66 it is shown that about $\frac{1}{2}$ day after labelling of the maternal plasma iron 1 gm of foetal liver contains 143 times more ^{59}Fe than does the same weight of the maternal liver. In the former about 80 per cent of the ^{59}Fe which passed the placenta accumulates, demonstrating conspicuously the fact that in the rabbit embryo the liver is the main haemopoietic organ. Exposure of the rabbit to 500 r increases the amount of ^{59}Fe passing the placenta to twice the value in non-exposed animal. This is a result of the higher plasma ^{59}Fe concentration of the maternal plasma in the exposed animal. (1959) It was recently found by us that the maternal ^{59}Fe which reaches the mouse foetus is much better conserved during life than ^{59}Fe injected into the mouse intraperitoneally. $\frac{1}{5}$ 0/0 per day is lost daily from the former, $\frac{1}{2}$ 0/0 from the latter

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67. RATE OF FORMATION OF NUCLEIC ACID IN THE ORGANS OF THE RAT

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THE rate of formation of nucleic acid of the thymus nucleic acid type was investigated in the organs of the rat by administering labelled phosphate to rats and by determining the labelled P content of the desoxyribose nucleic acid extracted from the organs after the lapse of some days. The percentage of labelled nucleic acid present indicates the percentage of the total nucleic acid of the organs which is built up in the course of the experiment, as described in this note.

Preliminary figures on the rate of formation of nucleic acid in some of the organs of the rabbit were communicated at an earlier date (HAHN and HEVESY, 1940). Data are furthermore available on the rate of formation of labelled "nucleoprotein" in some organs of the mouse (TUTTLE, ERF and LAWRENCE, 1941). In our previous work, we extracted the nucleic acid with sodium chloride solution. TUTTLE and his colleagues removed the acid soluble and the phosphatide P fractions from the organs investigated and considered the residual P to be phosphorus of the "nucleoprotein" fraction. Extended studies carried out in this laboratory lead to the result that it is hardly possible to obtain nucleoprotein sufficiently purified from non-nucleoprotein phosphorus by the last mentioned procedure. Muscles and other organs of the frog containing labelled P were treated for weeks daily alternately with trichloroacetic acid solution and with ether alcohol mixtures. The specific activity of the remaining "nucleoprotein" P was determined subsequently. It was found much higher than the specific activity (activity per mgm P) of phosphorus obtained from properly purified nucleic acid. As shown in this note, the rate of formation of nucleic acid in most organs is very slow and, correspondingly, the specific activity of the nucleic acid P few hours and even some days after the administration of labelled P is low also.

After the lapse of 2 hours, 1 mgm nucleic acid P of the liver of the rat, for example, contains but $2 \cdot 10^{-4}$ per cent of the labelled phosphorus administered, while the corresponding figure for 1 mgm acid soluble P of the liver is about 1 per cent. If only 10^{-3} part of the isolated nucleic acid P

is composed of acid soluble P present as an impurity, a grossly erroneous value will be found for the specific activity of the nucleic acid P, viz. $12 \cdot 10^{-4}$ instead of $2 \cdot 10^{-4}$. This example illustrates the necessity of an exceedingly careful purification of the nucleic acid fraction from all non-nucleic acid phosphorus. In our work, we are not faced with the great difficulties which were surmounted by HAMMARSTEN in his experiments which lead to the preparation of non-depolymerized nucleic acid. On the other hand, we have to avoid the presence of even minimal amounts of non-nucleoprotein P, the presence of which in any other but the radioactive investigations would certainly not be found disturbing.

EXPERIMENTAL PROCEDURE

We applied the method of extraction and purification described by KLEIN and BECK (1935) adapted to work with tissue containing radioactive phosphorus, as previously used by H. VON EULER and one of the present writers (1942) in their work on the rate of formation of nucleic acid in the Jensen sarcoma of the rat. The washed tissue is stirred with an equal weight of 5 per cent sodium chloride solution brought to boiling. Acetic acid is added until the major part of the proteins present is precipitated. Sodium acetate and sodium hydroxyde are then added and the alkaline solution is heated until the tissue is dissolved.

The next operation is carried out in a slightly acid solution. This is obtained by adding acetic acid. From this solution, the protein present is removed by adding a dialysed colloidal iron hydroxide solution containing 5 per cent F_2O_3 . An excess of acetic acid is added and the hot solution is filtered. By adding an equal volume of methylalcohol to the filtrate, the crude nucleic acid precipitates.

The crude nucleic acid is dissolved in sodium hydroxide and is precipitated with hydrochloric acid and methylalcohol. Before re-precipitating the nucleic acid, we added about 10 mgm $(NH_4)_2HPO_4$ for each mgm nucleic acid. By doing so, we diluted the free radioactive phosphate possibly present in the nucleic acid. If the crude nucleic acid carried before and after the precipitation 1 mgm free phosphate, the free phosphate will be but 1/100 as active after precipitation as previously. This procedure is repeated several times, and each time inactive $(NH_4)_2HPO_4$ is added to the alkaline solution.

The purification process entails a substantial loss of nucleic acid. However, it is not the total desoxyribose nucleic acid content of the organs in which we are interested, but the percentage of the desoxyribose nucleic acid content which is built up during the experiment, i. e. the rate of renewal of the nucleic acid molecules. We are interested in the activity of 1 mgm nucleic acid P and not in the activity of the total nucleic acid present in the organs.

The purified nucleic acid is brought into solution by wet ashing. $\frac{1}{5}$ is reserved for colorimetric P determination, while $\frac{4}{5}$ are precipitated as ammonium magnesium phosphate; the activity of the precipitate is determined. The interpretation of the activities of which are to be compared, have the same weight. To obtain this, a suitable amount (about 80 mgm) of Na_2HPO_4 is added to the solution before precipitating the ammonium magnesium compound.

An aliquot of the solution administered by subcutaneous injection is treated in the same way. If this "standard preparation" has, for example, 1/1000 of the activity administered and the nucleic acid fraction containing 1 mgm P has 1/100

of the activity of the standard preparation, we find 0.001 per cent of the labelled P administered to be present in 1 mgm nucleic acid P.

The weight of the male adult albino rats used varied between 250 and 320 gm. They were kept in a normal diet. The labelled phosphate administered by subcutaneous injection had an activity corresponding to 3 μ Curie.

CONTROL OF THE EFFECTIVITY OF THE PURIFICATION PROCESS

To a crude nucleic acid fraction containing 200 mgm nucleic acid we added 75,000 relative units of radioactive phosphorus (^{32}P). Each time, decreasing amounts of inactive ammonium phosphate were added to the filtrate containing the nucleic acid. The amount of $(\text{NH}_4)_2\text{HPO}_4$ added varied between 100 and 30 mgm. After successive purifications, the following activity figures were obtained for the nucleic acid.

Number of purifications	Activity of fractions
0	75,000
1	—
2	34
3	9

The nucleic acid purified 3 times thus contained but 1/8000 part of the labelled phosphate added.

The successive purification of nucleic acid P from other than free P can be controlled in the following way. The specific activity of an aliquot of the nucleic acid P of the purified sample is determined. Another aliquot of the sample is dissolved subsequently and is re-precipitated as described above, but, without adding phosphate. The specific activity of the P of the precipitate obtained is then again determined. If no other phosphorus than nucleic acid P is present in the sample the specific activities determined should be identical. When controlling the purity of nucleic acid extracted from the liver and purified twice in the manner described above, a further purification reduced the specific activity of the phosphorus obtained from the nucleic acid by 5.5 per cent.

Furthermore, we investigated whether the nucleic acid obtained is exclusively of desoxyribose type or contains also some nucleic acid of the ribose nucleic acid type.

To 7.6 mgm active thymus nucleic acid dissolved in 1.0 cc. $\frac{1}{10}$ N. NaOH about 60 mgm yeast nucleic acid, dissolved in 2 cc. $\frac{1}{10}$ N. NaOH were added. After precipitating but once, the nucleic acid, as described on p. 664, we redetermined the specific activity of the nucleic acid P. This was found to be 76 per cent of the specific activity of the value measured at the start of the experiment. A single precipitation sufficed thus to remove 96 per cent of the yeast nucleic acid added.

SPECIFIC ACTIVITY OF THE FREE P OF DIFFERENT ORGANS

The fact that greatly divergent figures are obtained for the specific activity of the nucleic acid fractions is due mainly to the highly different rate at which nucleic acid is formed in the different organs, molecules are built up. To calculate the rate of formation of nucleic acid, it is thus necessary to know the specific activity of the "free" phosphate present in the tissue cells. It does not suffice to know the specific activity of the free P at the end of the experiment. We have to determine this magnitude at different times in order to arrive at a value of the average specific activity of the free P during the experiment. The results obtained are seen in Table 1 which contains data on the specific activity of the "free" P extracted from the organs. They are obtained on rats killed at different times. In the case of the muscles,

TABLE 1. — SPECIFIC ACTIVITY OF THE FREE PHOSPHATE EXTRACTED FROM THE ORGANS⁽¹⁾

Organ	Duration of the experiment in hours								Average value of the specific activity during the experiment
	2	5	8.5	13	25	50	72	94	
Plasma	0.91	0.67	0.214	0.286	0.204	0.114	0.099	0.069	0.18
Liver	1.61	0.95	0.42	0.59	0.17	0.15	0.10	0.10	0.25
Kidney	1.10	0.78	0.43	0.57	0.18	0.16	0.096	0.10	0.24
Spleen	0.77	0.67	0.31	0.43	0.19	0.16	0.11	0.10	0.21
Mucosa of the small intestine	0.72	0.51	0.35	0.51	0.15	0.13	0.11	0.11	0.19
Muscle	0.27	0.13	0.12	0.38	0.062	0.084	0.090	0.055	0.11
Testes	0.13	0.12	0.086	0.14	0.062	0.079	0.072	0.06	0.080
Brain	0.044	0.052	0.045	0.083	0.047	0.057	0.053	0.051	0.054

⁽¹⁾ Specific activity usually denotes the activity of 1 mgm P in arbitrary units (often, the specific activity of the plasma P is taken = 100). The above specific activities denote the percentage of the ³²P administered present in 1 mgm P.

the free P was extracted from tissue samples taken from the narcotized rat. The tissue was placed in liquid air and extracted at once by grinding with 10 per cent trichloroacetic acid. This precaution has to be taken in order to avoid the decomposition of creatine phosphoric acid present in large amounts in the muscle. While, in experiments of long duration, as discussed below, the specific activity of the creatine phosphoric acid P does not differ from the specific activity of the free P, in experiments of shorter duration, however, large differences were found, and in these experiments a decomposition of creatine phosphoric acid prior to the removal of the free P would result in a lowering of the specific activity of the free P.

The specific activity of the creatine phosphoric acid was determined in the following way. After the removal of the free P as ammonium magnesium salt, the filtrate was slightly acidified and heated for a very short time. The free P obtained by the decomposition of creatine phosphoric acid was then again precipitated as ammonium magnesium salt. While, after the lapse of one day or more, the specific activity of the creatine phosphoric acid phosphorus was found to be just as high as the specific activity of the "free" P present in the muscle tissue, after the lapse of 2 hours the ratio of the specific activities was found to be only 0.6.

SPECIFIC ACTIVITY OF THE NUCLEIC ACID EXTRACTED FROM DIFFERENT ORGANS

The results of the determination of the specific activity of the nucleic acid phosphorus extracted from the different organs is seen in Table 2. I and II denote the values obtained in the first and second experiments respectively. In each experiment the organs of 8 rats were pooled. The values shown in the last column indicate the percentage ratio of the specific activity of the nucleic acid P and the specific activity of the "free" phosphate P of the different organs.

TABLE 2. — SPECIFIC ACTIVITY⁽¹⁾ OF THE NUCLEIC ACID PHOSPHORUS EXTRACTED FROM DIFFERENT ORGANS OF 8 RATS 4 DAYS AFTER THE ADMINISTRATION OF LABELLED PHOSPHATE $\times 1000$

Organ	Specific activity $\times 1000$		Percentage ratio of the specific activity of nucleic acid P and free P (Percentage renewal) ⁽²⁾
	I.	II.	
Small intestinal mucosa	12.7	15.4	59
Spleen	5.93	6.24	23
Muscle	1.05	1.42	8.8
Liver	1.01	1.66	4.2
Testes	0.83	0.97	10
Kidney	0.60	0.67	2.1
Brain	0.09	0.22	2.3

⁽¹⁾ Percentage of the ^{32}P administered present in 1 mgm P.

⁽²⁾ When calculating the above ratio, we must take into account that the nucleic acid has been extracted from the organs of 8 rats.

When calculating the figures of the last column, we have not taken the figures of the specific activity of the free phosphate P as stated in Table 1, but corrected these for the presence of labelled P in the extracellular space of the organs. The extracellular P is not utilized to build up nucleic acid, and we have to consider the ratio of the specific activity of the nucleic

acid P the specific activity of the cellular free P. The figures for the size of the extracellular space of the organs were taken from a paper by MANERY and HASTINGS (1939) and it was assumed that the labelled phosphate concentration of the extracellular fluid is identical with the labelled

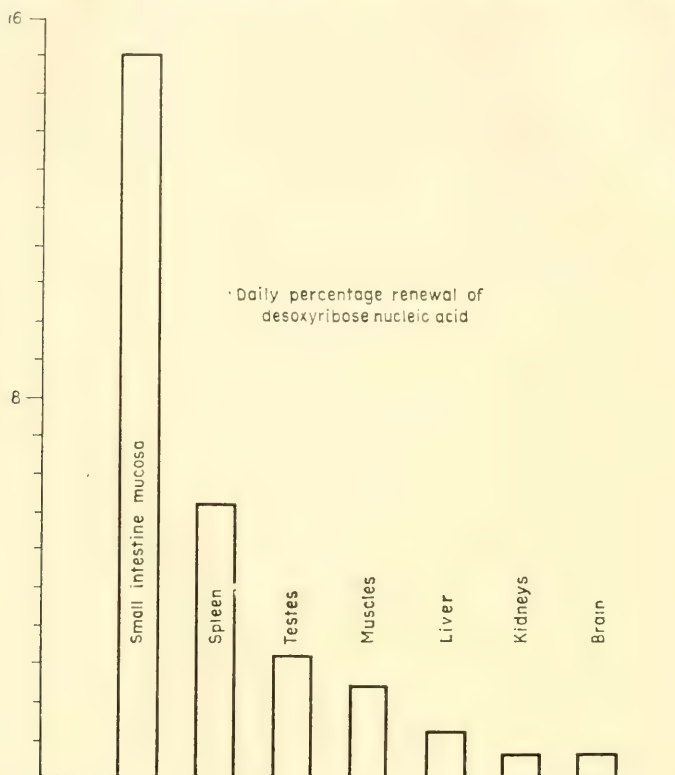


FIG. 1.

phosphate concentration of the plasma water. The correction for the presence of labelled phosphate in the extracellular space was largest for the testes, but even in this case only 12 per cent of the value stated in Table 1. We did not correct the specific activity found for the free P of the brain in view of the uncertainty prevailing as to size and composition of the extracellular fluid of the brain. Therefore, it is possible that the rate of renewal of the brain nucleic acid is not slightly larger, but smaller than the corresponding value found for the kidneys (cf. Table 2).

The percentage ratio of the specific activity of nucleic acid P and free P (the percentage renewal of the nucleic acid) in different organs is seen in Table 2 and Fig. 1. The highest percentage of new nucleic acid is found in the small intestine, while the lowest figure is shown by the brain. Remarkably low figures are found for the liver. In the

course of 1 hour, a very large part of the acid-soluble P compounds and a few per cent of the phosphatides present in the liver are renewed. Compared with these figures, the rate of renewal of nucleic acid in the liver is negligible.

The percentage ratio of the specific activity of the nucleic acid P and the free P indicates the percentage of new nucleic acid present, i.e. nucleic acid formed in the course of the experiment. We cannot state with certainty whether this new nucleic acid is formed in the organ in which it is found or transported from another organ in which it was built up. It would be conceivable that the nucleic acid molecules built up in the intestinal mucosa, for example, where we find far the greatest rate of renewal of nucleic acid, reach the circulation and are deposited in the muscles. Information on this point can be obtained on the same line or on similar lines on which the origin of the phosphatides present in the yolk was investigated (HEVESY and HAHN, 1938). The nucleoproteins are probably built up in the nuclei of the cells and not carried from organ to organ. The low new (labelled) nucleic acid content of the liver can be interpreted as an argument against the last mentioned interpretation. The liver takes up easily constituents present in the circulation and, if any organ takes up from the circulation nucleoproteins and thus nucleic acids, we would expect the liver to do so. The active nucleic acid content of the liver nucleic acid is, however, very low and this fact supports the view that the active nucleic acid molecules present in the liver are synthesized in this organ. The rate of renewal of the nucleic acid molecules in the liver may be identical with the rate of new formation of liver cells⁽¹⁾.

The figures for the rate of formation of nucleic acid in the organs of the rat found in this investigation are very much lower than those for the renewal of nucleic acid or of "nucleoproteins" by different experiments both in the organs of the rabbit and in the organs of the mouse. In the liver of the rabbit (HAHN and HEVESY, 1940), for example, 6 per cent of the nucleic acid present were found to be renewed in the course of 11.5 hours. In the liver of the mouse (TUTTLE, ERF and LAWRENCE, 1941), in the course of 6 hours, about 40 per cent of the "nucleoproteins" present were found to be labelled. In these experiments, the nucleic acid P and the "nucleoprotein P", respectively, contained presumably some strongly active acid-soluble or phosphatide phosphorus, the presence of which was presumably responsible for the high values obtained for the rate of renewal of the nucleic acid and the "nucleoproteins".

⁽¹⁾ The rate at which liver cells are renewed is not known. While this rate may be smaller than the rate of formation of nucleic acid in the liver cells, it can hardly be larger.

AMOUNT OF NUCLEIC ACID FORMED DAILY IN THE DIFFERENT ORGANS OF THE RAT

If we assume that the labelled desoxyribose nucleic acid found in an organ is synthesized in the organ in question, we can estimate from the data of Table 2 and the desoxyribose nucleic acid content of the organs the total amount of desoxyribose nucleic acid which is built up daily in the different organs. Data are available on the total nucleic acid content of the organs of the rat. These data are given in Table 3. With the exception of the figure stated for the nucleic acid content

TABLE 3. — UPPER LIMIT OF THE DESOXYRIBOSE NUCLEIC ACID CONTENT OF DIFFERENT ORGANS OF THE RAT

O r g a n	Desoxyribose nucleic acid content (mgm per gm)
Muscle	1.4
Heart	1.4
Brain	2.5
Kidney	3.3
Testes ⁽¹⁾	5.7
Mucosa of the small intestine	5.1
Liver	6.5
Spleen	10
Thymus ⁽²⁾	30

⁽¹⁾ Horse testes. (JAVILLIER and ALLAIRE, 1926.)

⁽²⁾ Horse thymus. For calf thymus, 36 was found. (JAVILLIER and ALLAIRE, 1926.)

of the intestinal mucosa, they are taken from a paper by JAVILLIER *et al.* (1928). These workers state the nucleic acid P content of the tissue investigated; we multiplied their figures by 12 to arrive at the nucleic acid content. As no data were available for the nucleic acid content of the mucosa of the intestine, we determined the desoxyribose nucleic content of the mucosa small intestine by using DISCHE's method (1930) in a slightly modified form, as applied by VOWLES (1940). This method is based on the fact that, when heating a solution of desoxyribose nucleic acid in the presence of diphenylamin, acetic acid and sulphuric acid, a violet colouring is obtained, the intensity of the colour being proportional to the concentration of the nucleic acid. As standard preparation we used a thymus nucleic acid preparation kindly presented us by Professor HAMMARSTEN. As the reaction used is not strictly specific for desoxyribose, the figure obtained has also to be considered an upper limit of the desoxyribose nucleic acid content of the intestinal mucosa.

If the bulk of the proteins present were not previously precipitated, the colorimetric determination gave a higher value (6.9 mgm). The same observation was made by VOWLES (1940).

The upper limit of the amount of desoxyribose nucleic acid built up daily in the different organs of the rat is given in Table 4.

TABLE 4. — UPPER LIMIT OF THE AMOUNT OF DESOXYRIBOSE NUCLEIC ACID BUILT UP DAILY IN THE DIFFERENT ORGANS OF RATS WEIGHING ON THE AVERAGE 275 GM

Organ	Weight in gm	Nucleic acid present in the organ in mgm	Upper limit of desoxyribose nucleic acid built up in the course of a day in mgm
Brain	1.43—1.53	3.7	0.02
Kidney	1.72—1.76	5.8	0.03
Testes	1.93—2.57	12.8	0.34
Spleen	0.86—0.81	8.3	0.48
Liver	9.24—9.06	57.5	0.61
Mucosa of the small intestine	4	21	3.0
Muscle	111—109	154	3.4

As already mentioned previously, when calculating the above figures we assume that the nucleic acid found in an organ is built up in the organ. This assumption may not hold strictly, as the blood contains some nucleic acid which may have been carried from the organs into the circulation. However, this amount is small. Assuming the rat blood to contain the same nucleic acid concentration as human blood which, according to JAVILLIER and ALLAIRE (1931) amounts to 0.3 mgm per gm blood, the total amount of desoxyribose + ribose nucleic acid present in the circulation of a rat weighing 275 gm amounts to about 6 mgm, thus about $\frac{1}{50}$ of the total nucleic acid content of the rat.

Summary

Labelled phosphate is administered to adult rats by subcutaneous injection. After the lapse of 4 days, the rats are killed and the desoxyribose nucleic acid present in different organs is extracted. By comparing the activity of 1 mgm desoxyribose nucleic acid P with the activity of 1 mgm cellular inorganic P of the same organ, data on the percentage renewal of the desoxyribose nucleic acid present in the organs are obtained.

In the course of 4 days, only a minor part of the desoxyribose nucleic acid present in all the organs investigated is found to be labelled, i. e. formed in the course of the experiment. The greatest daily formation of nucleic acid (15 per cent) takes place in the mucosa of the small intestine. This is followed by the spleen (5.8 per cent), the testes (2.6 per cent), the muscles (1.9 per cent), and the liver (1.0 per cent). The lowest figures are shown by the kidneys and the brain (0.6 per cent).

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68. RATE OF RENEWAL OF RIBO- AND DESOXYRIBO NUCLEIC ACIDS

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ENZYMIC processes coupled with phosphorylation often take place at a remarkably rapid rate. A large percentage of the molecules of many of the acid-soluble phosphorus compounds and, to a minor extent, also those of phosphatides present in the liver and some other organs are renewed within a short time. This is demonstrated by the observation that shortly after the administration of ^{32}P these molecules are found to contain labelled phosphate.

That the presence of ^{32}P in the molecules of organic phosphorus compounds indicates an enzymic synthesis of such molecules is most strikingly demonstrated by a recent experiment of CHAIKOFF and his associates (1942). These authors have shown that labelled phosphatides are formed when surviving liver slices are shaken with bicarbonate Ringer solution containing labelled phosphate, that this formation is, however, impaired in the absence of oxygen, and homogenized liver tissue completely loses its ability to incorporate ^{32}P into the phosphatide molecule. A non-enzymic process could hardly be dependent on the intactness of the tissue cells.

In contradistinction to the above mentioned compounds, as found in previous (HEVESY and OTTESEN, 1943 ; AHLSTRÖM, EULER and HEVESY, 1944 ; BRUES, TRACY and COHN, 1944) and in the present investigations, desoxyribo nucleic acid molecules present in the liver are at a very slow rate only. This result falls in line with the view that desoxyribo nucleic acid is present in the nuclei of the cells and is involved in the process of cell division. As the mitotic process in the liver of a fully grown rat takes place at a very slow rate only, the low rate of formation of desoxyribo nucleic acid in the fully grown liver is in no way surprising, neither is the much higher rate of formation observed in the liver of the only few days old rat. The rate of formation on new desoxyribo nucleic acid molecules present in the liver of 3 to 4 days old rats was found to be about 20 times that of the corresponding figure in outgrown rats (AHLSTRÖM, EULER and HEVESY, 1944).

In contradistinction to the desoxyribo nucleic acid, a large part of ribo nucleic acid is located in the cytoplasm and, according to the view developed by CASPERSSON (1940), is involved in protein synthesis. As such a synthesis takes place at a marked rate in the liver, the rate of renewal of the ribo nucleic acid can be expected to be larger than the rate renewal of desoxyribo nucleic acid in this organ. The present note contains the results of experiments in which the rate of renewal of both types of nucleic acid was determined, viz. in the liver, the spleen, and the intestinal mucosa of the rat and also in the total rat body. The methods applied in the isolation of the nucleic acids will shortly be published by one of the authors.

RESULTS

If we assume the free phosphate to be built into the nucleic acid molecules without the formation of an intermediary phosphorus compound of comparatively long life, the ratio of the specific activity of the nucleic acid P and the free P is a measure of the rate of formation of new nucleic acid molecules and thus of the extent of renewal of such molecules. If, previous to the formation of labelled nucleic acid molecules, labelled precursors would be built up at a rate slower than the rate of formation of new nucleic acid molecules, the ratio of the specific activity of the nucleic acid P and that of the free P would no longer be a proper measure of the rate of renewal of the nucleic acid. In the latter case, namely, during part of or possibly throughout the whole experiment, new nucleic acid molecules would be built up without participation of ^{32}P . The participation of such labelled intermediary compounds of a very long life in the formation of desoxyribo nucleic acid in the liver is, however, highly improbable in view of the comparatively short lifetime of most of the acid-soluble phosphorus compounds and the very long lifetime of desoxyribo nucleic acid molecules present in the liver.

In our experiments the specific activity of the nucleic acid P is compared with the specific activity of the free P at the end of the experiment. As the specific activity of the free P changes throughout the experiment, the specific activity of the nucleic acid P at the end of the experiment should, however, be compared with the mean specific activity of the free P during the experiment. As the liver takes up ^{32}P at a very rapid rate, and its free ^{32}P content culminates within the first 2 hours, the end value and the average value of the specific activity of the free liver P do not differ essentially, the average value being about 5 per cent lower than the end value (AHLSTRÖM, EULER and HEVESY, 1944). In the case of the spleen the corresponding figure is about 25, and even larger differences are found in the case of the intestinal mucosa. The figures

of columns 2 and 3 of Table 1 should therefore be multiplied by 1.05 in the case of the liver, for example, to obtain a correct value for the amount of the rate of renewal of the desoxyribo nucleic acid present in the liver. In the figures of Table 1 we have not introduced this correction, as we are mainly interested in the relative rate of renewal of the desoxyribo and ribo nucleic acids.

Table 1 contains the results of an experiment in which three rats in nitrogen-equilibrium weighing 252, 182 and 215 gm were injected subcutaneously with respectively 7.5, 6.6 and 5.6 microcuries of ^{32}P per 100 gm body weight.

After 2 hours the animals were killed and the organs prepared according to a method which will soon be published by one of the authors.

TABLE 1. — RATIO OF THE RATE OF FORMATION OF THE RIBO NUCLEIC ACID AND DESOXYRIBO NUCLEIC ACID IN THE ORGANS OF THE RAT IN THE COURSE OF 2 HOURS

O r g a n	Percentage ratio of the specific activity of the nucleic acid P and that of the free P				Ratio of the rate of formation of ribo and desoxyribo nucleic acid
	Ribo		Desoxyribo		
	nucleic acid				
Liver	3.3;	3.6	0.12;	0.09	33
Spleen	3.1;	10.2	2.2;	2.2	3
Intestine	7.1	4.1	3.4;	2.3	2

As recorded in Table 1, the rate of renewal of ribo nucleic acid in the liver is as much as 33 times larger than the rate of renewal of desoxyribo nucleic acid. In spite of the finding that ribo nucleic acid is renewed at an even larger rate in the spleen and the intestinal mucosa than in the liver, the ratio of the rate of renewal of ribo- and desoxyribo-nucleic acids in these organs is only 3 and 2, respectively. This low ratio is due to the comparatively high rate of formation of desoxyribo nucleic acid in these organs. From the above figures it follows that the rate of renewal of both types of nucleic acid is highest in the intestinal mucosa and in the spleen.

The specific activity of both the desoxyribo and the ribo nucleic acid P extracted from the rat liver was determined by BRUES, TRACY and COHN (1944) in experiments lasting 3 to 8 days. In these experiments the ribo nucleic acid P was found to be only 5 to 6 times as active as the desoxyribose nucleic acid P. The discrepancy between these figures and those obtained by us may be due, at least mainly, to the much longer duration of the last mentioned experiments.

Specific Activity of the Nucleic Acid Phosphorus Extracted from the Total Rat

In another experiment the specific activity of both the total desoxy-ribo nucleic acid P and the total ribo nucleic acid P extracted from a rat weighing 194 gm was determined. The activity of labelled sodium phosphate amounted to 8.1 microcuries per 100 gm animal weight. The time of the experiment was 2 hours. The results of this experiment are seen in Table 2.

TABLE 2. — SPECIFIC ACTIVITY OF THE NUCLEIC ACID P EXTRACTED FROM THE TOTAL RAT COMPARED WITH THE CORRESPONDING VALUES FOR LIVER, SPLEEN, AND INTESTINAL MUCOSA. THE VALUE FOR THE SPECIFIC ACTIVITY OF THE TOTAL RAT RIBO P IS ASSUMED TO BE = 100

S a m p l e	Specific activity		
	Ribose	Desoxyribose	Free P
	nucleic acid		
Total rat	100	60	
Liver	164	4.4	5100
Spleen	292	63	2850
Intestine	112	63	2770

As shown in Table 2, the specific activity of the average nucleic acid P of the rat is almost identical with the corresponding value of the ribo and desoxyribo nucleic acid, respectively extracted from the intestine.

The interpretation of the significance of the specific activity figures obtained for the total rat encounters some difficulties, as the specific activity of the free P utilized in the formation of the labelled nucleic acid molecules is unknown. If the specific activity of the free P utilized in building up the average body nucleic acid would correspond to the specific activity of the free liver P, the percentage "rate of renewal" of the body ribo and desoxyribo nucleic acids would be 2.0 and 1.2, respectively. If the specific activity of the free P utilized in building up the average nucleic acid of the organism would correspond to the specific activity of the intestinal P, larger figures, i.e. 3.6 and 2.2, respectively, would be obtained.

When calculating the last mentioned figures, we compared the specific activity of the nucleic acid P at the end of the experiment with the specific activity of the free intestinal P at the end of the experiment. Correctly we should have considered the mean value of the specific activity of the three intestinal P prevailing during the experiment. The mean

value of this magnitude is about almost half of its end value, we have therefore to multiply the figures mentioned above (3.6 and 2.2 respectively) by about 2 to obtain an approximate value of the percentage renewal of the ribo- resp. desoxyribo nucleic acid in the course of 2 hours.

It is improbable that a so highly active free phosphate is utilized in the building up of the nucleic acid molecules as found in a 2 hours experiment in the liver. Liver and kidneys have a privileged position concerning the rate of intrusion of phosphate. The amount of nucleic acid present in the liver and the kidneys makes out, furthermore, only a small percentage of the total nucleic acid content of the organism. It is much more probable that free P of similar specific activity as found in the intestine is applied in the building up of the labelled nucleic acid molecules. In fact, the amount of nucleic acid present in the mucosa of the digestive tract makes out a large percentage of the body nucleic acid. While the body nucleic acid contains also slightly radioactive fractions, viz. those originating from the liver, the kidneys, and the brain and fractions of restricted radioactivity originating from the muscles (HEVESY and OTTESEN, 1943), it contains also fractions of higher activity than found in the intestinal mucosa, viz. those originating from the bone marrow, the thymus and lymph nodes (ANDREASEN and OTTESEN, 1944, 1945). The lymphocytes secreted into the organism can also be expected to contain pronouncedly active nucleic acid. This makes it understandable that the rate of renewal of the average body nucleic acid corresponds to about the rate of renewal of the intestinal nucleic acid and is thus quite pronounced for both types of nucleic acid in contradistinction to the rate of renewal found in the liver, which is very low in the case of desoxyribo nucleic acid and appreciably higher in the case of ribose nucleic acid.

DISCUSSION

In view of the high desoxyribo nucleic acid content of the lymphocytes and because they are partly produced in the spleen, the comparatively high rate of turnover of desoxyribo nucleic acid in the spleen is in agreement with our expectance.

As to the high figures found for the rate of renewal of desoxyribo nucleic acid in the intestinal mucosa, those are presumably due to the rapid new-formation of cells mechanically destroyed in the course of the digesture success.

If we accept the view put forward by CASPERSSON, the high rate of renewal of ribo nucleic acid is in no way surprising. The high figures found for the rate of renewal of ribo nucleic acid in the intestine, the spleen and the liver is just what we would expect in view of the impor-

tance of these organs in protein metabolism. The incorporation of labelled sulfur into protein sulfur is found to be higher in the intestine than in any other organ (TARVER and SCHMIDT, 1942) and the ^{15}N content of the proteins isolated from the intestinal wall of the rat after administration of isotopic l(—)-leucine is larger than the corresponding value for any other organ investigated. Somewhat smaller values for the ^{15}N content of the proteins isolated from the spleen were found, and still smaller values for the ^{15}N of the proteins isolated from the liver (SCHOENHEIMER, RATNER and RITTENBERG, 1939). The rate of formation of ribose nucleic acid in these three organs diminishes in the same sequence.

If we want to state, not as above the percentage, but the amount of nucleic acid formed during the experiment, we must know the nucleic acid content of the organs of the rat and of the total rat.

Some preliminary figures for the total nucleotide P of the liver, spleen, intestine and total rat and also some preliminary figures for the share of polydesose and polyribo nucleotides in the total nucleotides is seen in Table 3. The method applied in obtaining these figures and more accurate data will be shortly published by one of the authors.

TABLE 3. — POLYDESOSE NUCLEOTIDE PHOSPHORUS AND POLYRIBO NUCLEOTIDE PHOSPHORUS CONTENT OF SOME ORGANS AND OF THE TOTAL RAT

	Approximate share of poly- desose nucleotides in the total nucleotides %	gm nucleotide P per 100 gm dry weight	gm polydesose nucleotide P per 100 gm dry weight	gm polyribo- nucleotide P per 100 gm dry weight
Total rat	45—50	0.232	0.11	0.12
Liver	35	0.350	0.12	0.23
Spleen	75	0.643	0.48	0.16
Intestine	57	0.669	0.38	0.29

Assuming the percentage formation of the polydesose nucleic acid of the total rat in the course of 2 hours to be 4 (cf. p. 676) and the fresh weight of the rat to amount to five times its dry weight, in a 200 gm rat in the course of 2 hours about 2 mgm polydesose nucleotide P will be incorporated. The corresponding figure for the polyribo nucleotide P works out to be 3. In the total rat the rate of formation of the 2 types of polynucleotides does thus not differ very appreciably.

A very different result is obtained when comparing the amount of polydesose and polyribo nucleotide phosphorus incorporates in the liver. The figures work out, assuming the liver to weigh 6 gm, to be 0.0017 mgm and 0.094 mgm respectively. Fifty-five times more polydesose nucleotide than ribonucleotide is thus formed in the liver during the same time.

Assuming the spleen to weigh 0.8 gm, both the amount of polydesose nucleotide P and that of polydesose nucleotide P formed and still present in the spleen works out to be about 20 microgram.

Summary

Labelled sodium phosphate is administered to rats and after the lapse of 2 hours the specific activity of the ribo-nucleic acid phosphorus and that of the desoxyribo-nucleic acid phosphorus determined.

In the liver the specific activity of the ribo-nucleic acid P is found to be 33 times larger than the specific activity of the desoxyribo-nucleic acid P. In the course of 2 hours about 0.1 and 3.3 per cent respectively of these compounds were formed.

In the intestine and in the spleen in which the specific activity of the desoxyribo-nucleic acid is found to be about 20 times larger than the corresponding value in the liver, the specific activity of the ribo-nucleic acid phosphorus is only 2 to 3 times larger than the corresponding value of the desoxyribo-nucleic acid phosphorus.

The ribo- and the desoxyribo-nucleic acid phosphorus extracted from the total rat have a very similar specific activity to the corresponding phosphorus extracted from the intestine. In the total rat the difference in the rate of formation of the two types of nucleic acid is not very pronounced. In a rat weighing 200 gm approximately about 2 mgm desoxyribo-phosphorus and 3 mgm ribonucleic acid phosphorus are incorporated in the course of 2 hours.

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69. TURNOVER OF RIBOSENUCLEIC ACID IN THE JENSEN-SARCOMA OF THE RAT

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In previous papers the rate of formation of labelled desoxyribosenucleic acid in the Jensen-sarcoma in rats upon the administration of labelled phosphate was investigated.

In the present paper the results of investigations into the rate of renewal of the ribosenucleic acid in the Jensen-sarcoma of the rat will be reported.

EXPERIMENTAL

A few microcuries of labelled phosphate of negligible weight dissolved in 0.1 ml physiological sodium chloride solution were subcutaneously injected into rats (weighing 125–165 gm) inoculated with Jensen-sarcoma. Two hours after the injection the rats were sacrificed. The blood plasma and the sarcoma were secured and the inorganic phosphate was extracted with a 7 percent CCl_3COOH solution from both the plasma and an aliquot of the sarcoma tissue. The specific activity of the nucleic acid was determined on the greatest part of the sarcoma tissue, a minor part being applied in the determination of the specific activity of the inorganic P. The first mentioned determinations were made by the method described by SCHMIDT and THANHAUSER⁽¹⁾. This method is based on the assumption that after thorough extraction with trichloroacetic acid and ether, alcohol, chloroform and methanol the nucleic acids are the only phosphorus compounds present in the tissue. If the tissue purified in this way is dissolved in 1N NaOH solution at 37°, the desoxyribosenucleic acid remains unchanged, while the ribosenucleic acid is split into mononucleotides⁽²⁾. From the alkaline solution, CCl_3OOH precipitates desoxyribosenucleic acid and the ribosenucleotides are present in the filtrate. Then the desoxyribose and the ribose fractions were ashed and

⁽¹⁾ G. SCHMIDT and S. J. THANHAUSER, *J. Biol. Chem.* **161**, 83 (1945).

⁽²⁾ G. SCHMIDT, R. ARBILES, B. H. SWARTZ and S. J. THANHAUSER, *J. Biol. Chem.* **170**, 760 (1947).

an aliquot of the solutions obtained was used for the colorimetric determinations and another aliquot for the determinations of the radioactivity. 40 mgm Na_2HPO_4 were added to the aliquot, which was used for the determinations of the radioactivity, and then the phosphorus was precipitated as magnesium-ammonium phosphate.

As already observed by SCHMIDT and THANHAUSER and as shown below, the ribonucleic fractions always contain some inorganic P which possibly originates from some protein phosphorus present in the tissue and decomposed in the alkaline solution. The presence of inorganic P might also be explained by assuming that despite thorough extraction either some inorganic P or organic P compound was left in the tissue, which are identified as inorganic P in the alkaline solution of the tissue. It is also possible that a minute amount of nucleic acid P is split off in the alkaline solution.

The inorganic P present in an aliquot of the solution of ribosenucleotides was determined by the method of DELORY⁽¹⁾. Owing to the fact that large amounts of proteins are present in the solution containing the ribosenucleotids it was not possible to use the conventional methods for determining P. When calculating the specific activity of the ribosenucleic acid P from the inorganic P content and the activity of the ashed aliquot of the final solution, it must be taken into account that the whole amount of inorganic P present does not originate through ashing of the ribosenucleic acid P, because part of it is a remnant of the inorganic P, which has been present before ashing (see above). We have to deduct the values corresponding to the contaminating P both from the activity measured and from the inorganic P colorimetrically determined in the ashed ribosenucleic P fraction.

Since the total activity of the last mentioned component is mostly much lower than the activity of the ribose P fraction, the corrected values do not differ much from the non-corrected ones.

The following example illustrates the method used in determining the specific activity of nucleic acid P. Three sarcomata of an aggregate weight of 17.6 g were pooled. The minced tissue was extracted with ice-cooled 7 per cent trichloroacetic acid. The solution was filtered off through a Büchner funnel covered by a thick layer of hyflo. The tissue was repeatedly washed with 1 per cent CCl_3COOH . The last wash water did not show any colouring of amidol. The total value of 1 per cent CCl_3COOH added amounted to 800 ml. Trichloroacetic acid present in the tissue was quantitatively removed by washing with water. The tissue was then refluxed with 40 volumes of absolute alcohol for 14 hours. The ether — dried tissue was again extracted with 20 volumes of 7 per cent CCl_3COOH and the procedure described above repeated. After having been dried in vacuum, the tissue was refluxed with 3 : 1 alcohol-ether mixture for 11 hours. After removing the alcohol by washing with ether and drying in vacuum, the tissue was a third time

⁽¹⁾ DELORY, *Biochem. J.* **32**, 1161 (1938).

extracted with CCl_3COOH . The washed and dried tissue was then refluxed with a chloroform-methanol mixture (3:1) for 12 hours. After another extraction with CCl_3COOH , the extract had a negative reaction, which indicated the absence of inorganic P. After one more extraction with CCl_3COOH and chloroform-ether the tissue was dissolved in 180 ml. KOH by keeping it at 37° for one night. Only the hyflofilter was not dissolved. It was removed by centrifugation and the centrifugate was brought up to 200 ml. In order to be able to study the effect of repeated precipitation of desoxyribosenucleic acid two halves of this volume were treated separately. 5 per cent CCl_3COOH and 20 ml 0.2 N HCl were added to each solution.

From the 2 desoxyribosenucleic acid precipitates obtained, one (A) was dissolved in 1 N KOH solution and reprecipitated and ashed, while the other (B) was ashed after the first precipitations. The filtrate containing the ribosenucleotides, obtained after precipitation of the desoxyribosenucleic acid, was neutralized and brought to 300 ml. After neutralization a minor precipitate was thrown down. This precipitate was found to be only slightly radioactive. The total P of the neutralized solution was ashed and its P content (total P) determined. A larger aliquote, i.e., 125 ml, was used to determine the inorganic P content of the solution. The method of DELORY was used and 2 ml conc. NH_3 + 10 ml 2.5 per cent CaCl_2 + 10 ml 0.5 per cent MgCO_3 were added to the solution. The precipitate obtained was centrifuged, washed with 2 per cent NH_3 and dissolved in a 10 per cent CCl_3COOH solution. An aliquote was used for the colorimetric determination and from another aliquote, to which 80 mgm inactive Na_2HPO_4 had been added, P was precipitated as magnesiumammonium phosphate.

RESULTS

TABLE 1. — VALUES OBTAINED FOR THE PHOSPHORUS CONTENT OF FRACTIONS

Sample	P content in mgm	P content per gm tissue in mgm
Plasma (3 ml)	0.165 (inorganic)	0.055 (inorganic P per ml plasma)
Sarcoma tissue (0.593 gm).....	0.265	0.447
<i>A. (reprecipitated)</i>		
Desoxyribosenucleic acid	4.425	50.2
Total P in filtrate obtained after removal of desoxyribose- nucleic acid	7.425	
Inorganic P in filtrate	0.3087	
Ribosenucleic acid	7.1163	80.6
Etheric phase	0.0026	
<i>B. (not reprecipitated)</i>		
Desoxyribosenucleic acid	5.375	61.1
Total P in filtrate, obtained after removal of desoxyribose- nucleic acid	7.80	
Inorganic P in filtrate	0.327	
Ribosenucleic acid P	7.473	84.7
Etheric phase	0.0071	

Ribosenucleic acid P = total P in filtrate — inorganic P in filtrate.

To ascertain if the filtrates containing the ribosenucleotides are free of phosphatides, we were shaking the two filtrates (A and B) with 2 volumes of ether and 0.2 volume of N 0.1 HCl to extract phosphatides present. As seen above, the etheric phases contained a negligible amount of P only.

TABLE 2. — RESULTS OF ACTIVITY MEASUREMENTS
(^{32}P Content of Fractions)

Sample	Counts/min. of aliquote precipitated	Counts/min. of total volume	Counts/min. per mgm P
Plasma inorg. P	461.5	1445	8758
Sarcoma inorg. P	597.5	1792.5	6766
<i>A.</i>			
Desoxyribosenucleic acid P	260.5	651.3	147
Total P in filtrate after removal of desoxy- ribosenucleic acid	96.3	2890	389
Inorganic P in filtrate	126.9	846	2745
Ribosenucleic acid P	—	204.4	288
Residual fraction	11.7	14.6	—
<i>B.</i>			
Desoxyribosenucleic acid P	290.8	727	135
Total P in filtrate obtained after removal of desoxyribosenucleic acid	121.2	3030	398
Inorganic P in filtrate	101.5	951	2910
Ribosenucleic acid P	—	2079	279
Residual fraction	57.1	71.4	—

Specific Activity Ratios

("Percentage New-formation Figures")

Activity of 1 mgm desoxyribonucleic acid in percentage of activity of 1 mg:

A. Sarcoma inorganic P	2.18
B. Sarcoma inorganic P	2.0
A. Plasma inorganic P	1.68
B. Plasma inorganic P	1.55

Activity of 1 mgm ribosenucleic acid in percentage of activity of 1 mgm :

A. Sarcoma inorganic P	4.27
B. Sarcoma inorganic P	4.13
A. Plasma inorganic P	3.29
B. Plasma inorganic P	3.19

Ratio of the specific activities of $\frac{\text{ribosenucleic acid P}}{\text{desoxyribosenucleic acid P}}$

A 1.96

B 2.06

TABLE 3. — "PERCENTAGE FORMATION" OF NUCLEIC ACIDS IN THE JENSEN-SARCOMA OF THE RAT⁽¹⁾
(Time = 2 hours)

No. of Experiment	Activity of 1 mgm nucleic acid P in per cent of the activity of 1 mgm sarcoma inorganic P		Activity of 1 mgm nucleic acid P in per cent of the activity of 1 mgm plasma inorganic P		Ratio of the specific activities of ribosa P and desoxyribose P
	ribose	desoxyribose	ribose	desoxyribose	
232	5.38	1.79	4.23	1.33	3.0
233 A	4.27	2.18	3.29	1.68	2.0
233 B	4.13	2.0	3.19	1.54	2.1
234	5.62	1.53	5.78	1.57	3.7
235	3.88	1.90	4.04	1.98	2.0
236 ⁽²⁾	3.72	1.74	3.24	1.51	2.1 (fasting
237	3.55	1.60	3.75	1.09	2.3 rats)
238	2.74	1.76	5.0	1.77	1.6
239	7.71	2.73	5.0	3.2	2.8
Average value	4.6	1.9	4.2	1.7	2.4

Ratio of the ³²P content of ribosenucleic acid and desoxyribosenucleic acid in the Jensen-sarcoma = 2.4

⁽¹⁾ We came to the "Percentage Renewal" figures, contained in the above Table by comparing the end values of the specific activities of the nucleic acid P and inorganic, P. Correctly, we have to consider the mean value of the specific activity of the inorganic P during the experiment. The correction to be applied to arrive at correct figures is discussed on p. 685.

⁽²⁾ In this experiment the specific activity of the pyrophosphate P was determined as well. The ratios of the specific activities of pyrophosphate P : plasma inorganic P : sarcoma inorganic P was 1 : 1.10 : 0.97.

Similar values for the rate of formation of ribosenucleic acid and of desoxyribosenucleic acid were obtained in a number of other experiments, the result of which are summarized in Table 3. The average value, obtained for the percentage new-formation of desoxyribosenucleic acid, 1.9, compares fairly well with the average value, obtained in our previous investigations (2.05, 2.17), which involved a very large number of determinations.

In all our previous work⁽¹⁾ the desoxyribosenucleic acid P was purified from contaminating phosphorus compounds by repeated alternative boiling with conc. NaOH and reprecipitation of the dissolved desoxyribosenucleic acid P with methanol, containing HCl. This method has the disadvantage that a poor yield is obtained. The method of SCHMIDT

⁽¹⁾ H. EULER and G. HEVESY, *Kgl. Danske Videnskab. Selskab. Biol. Medd.* 17, No. 8 (1942).

and THANHAUSER has the advantage that it permits a quantitative or almost quantitative isolation of desoxyribosenucleic acid and can thus be carried out even if only small tissue samples are available.

The Average Value of the Specific Activity of the Inorganic P in the Sarcoma during the Experiment

The specific activity of the cellular inorganic P of the sarcoma depends on the rate (a) at which the labelled phosphate reaches the surface of the cells, (b) at which it penetrates into the cells of the tissue, (c) at which it is incorporated into organic P compounds in the cells (the incorporation of the inorganic ^{32}P into organic compounds goes hand in hand with the corresponding formation of inactive phosphate by the degradation of the inactive organic compounds present in the cells of the tissue). The magnitudes of these rates varies between the sarcomata.

When comparing the specific activities of the inorganic P fractions extracted from fresh and necrotic sarcoma 2 hours after the administration of ^{32}P tissue, we found previously⁽²⁾ that the values determined in the fraction of necrotic sarcoma were roughly only half the magnitude of those computed in the fraction extracted from fresh sarcoma. Also, the values obtained for the specific activity of inorganic P extracted from different parts of the fresh sarcoma tissue varied widely⁽²⁾. In the experiments here reported, each rat was first inoculated with Jensen-sarcoma and 15 days later 0.1 ml physiological sodium chloride solution, containing a negligible amount of phosphorus of about $\frac{1}{2}$ microcurie activity was administered to each rat. The 12 rats were sacrificed $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 hours respectively after the administration of ^{32}P . The ratio between the end values and the average value of the specific activities of the tissue inorganic P was found to vary between 1.17 and 1.57. In view of these variations it would be advisable to determine in each case the average specific activity of the sarcoma inorganic P. This presents great difficulty as such determinations would necessitate to secure samples from the same sarcoma at different dates. We determine therefore usually only the end value of the specific activity of the sarcoma inorganic P and assume this to be 1.3 times the average value. We arrive at the last mentioned figure by taking the average of the results obtained in experiments as described above. Correspondingly, we have to multiply the values of the "percentage new-formation", obtained by comparing the end values of the specific activity of the nucleic acid P with the specific activity of the end-value of the inorganic P, by 1.3 to arrive at the correct value of the percentage renewal of the sarcoma nucleic acids in experiments lasting 2 hours (cf. Table 3 in which the

⁽²⁾ H. EULER and G. HEVESY, *Ark. Kemi A* **17**, No. 30 (1944).

uncorrected values of the "percentage renewal" are set out). The ratio between the average and end-value of the specific activity of the sarcoma inorganic P can vary appreciably from sarcoma to sarcoma. These variations influence unfavourably the accuracy of the determination of the percentage new-formation of the nucleic acids in the sarcoma.

It may be assumed that the increase in the ribosenucleic acid content of the growing sarcoma during the course of the experiment (2 hours) amounts to about the same value as the increase of the desoxyribose-nucleic acid, i.e., 0.9 per cent of the amount present. Out of the 6 per cent newly formed ribosenucleic acid during the experiment, $\frac{1}{6}$ is thus due to additional formation of ribosenucleic acid, while $\frac{5}{6}$ are due to renewal of old molecules.

The value of the rate of renewal of ribosenucleic acid in the Jensen-sarcoma is about twice of the corresponding value (3.5) determined in the liver.

The Nucleic Acid P Content of the Jensen-Sarcoma

From the colorimetric determination of the P of the desoxyribose-nucleic acid and the ribosenucleotides we computed the values set out in Table 4.

The ratio between the desoxyribose and ribosenucleic acid of the Jensen-sarcoma is appreciably smaller than that between those in liver and most other organs. This is mainly due to the large desoxyribose-nucleic acid content of the sarcoma tissue.

TABLE 4. — NUCLEIC ACID P
CONTENT OF JENSEN-SARCOMA

mgm% in fresh tissue	
Desoxyribose P	Ribose P
42	64
54	87
65	91
46	77
36	52
57	63
50	68
52	78
41	58
52	67
57	67
Mean value 50	86

$$\text{Ratio } \frac{\text{Ribose P}}{\text{Desoxyribose P}} = 1.7$$

Summary

Radiophosphorus is injected into full-grown rats inoculated with Jensen-sarcoma. The specific activity of 1 mgm ribonucleic acid extracted from the Jensen-sarcoma at the end of 2 hours from the beginning of the experiment was found to be 6 per cent of the average specific activity of the inorganic P of the sarcoma throughout the experiment. On the basis of the assumption that the precursor of the ribonucleic acid P is either the cellular inorganic P of the sarcoma or organic P which is rapidly brought in exchange equilibrium with the cellular inorganic P, the above figure represents the percentage of the new-formation of the ribonucleic acid present in the Jensen-sarcoma in the course of 2 hours.

Out of 6 labelled ribonucleic acid molecules 1 represents additional formation due to growth of the sarcoma during the experiment, while the formation of roughly 5 new molecules is compensated by the simultaneous disappearance of 5 "old" ribonucleic acid molecules.

The ratio between the specific activity of the ribonucleic acid P and the specific activity of the desoxyribonucleic acid P of the Jensen-sarcoma is 2.4.

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70. LIFE-CYCLE OF THE RED CORPUSCLES OF THE HEN

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THE life-cycle of the mammalian red corpuscles is not known with certainty. Values varying between 30 and 200 days are recorded. One would expect the problem to be easily solved by making use of an isotopic indicator, that is, by labelling the corpuscles. In trying to find a suitable indicator, great difficulties are encountered due to the fact that almost every compound present in the corpuscles is renewed at a comparatively rapid rate. Only such labelled molecules which have a longer life-time than the red corpuscles in which they are located can be used as indicators. Iron atoms incorporated with haemoglobin molecules remain unchanged during the life-time of the red corpuscles⁽¹⁾. HAHN and his colleagues⁽²⁾, however, found that the iron atoms contained in the debris of the haemoglobin of decayed corpuscles are preferentially used in the formation of new corpuscles. This fact makes radioactive iron unsuitable for the determination of the life-cycle of the red corpuscles.

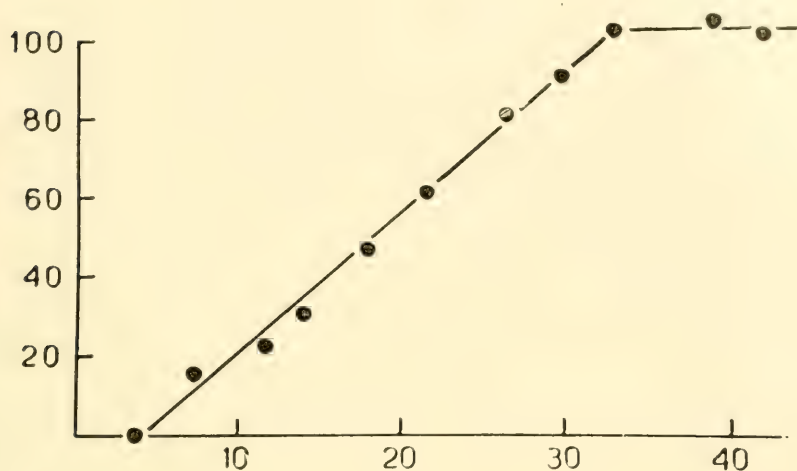
We found desoxyribose nucleic acid phosphorus to be a suitable indicator for the determination of the life-cycle of nucleated corpuscles. In contradistinction to desoxyribose nucleic acid molecules present in some organs, those found in the red corpuscles of the hen are not renewed at an appreciable rate. In experiments *in vitro*, in which hen blood was shaken in an oxygen atmosphere in the presence of labelled sodium phosphate, no active desoxyribose nucleic acid was found to be formed, in contradistinction to other active phosphorus compounds. Furthermore, activity was absent in the desoxyribose nucleic acid present in the circulating red corpuscles of the hen up to four days after administration of radioactive phosphate.

Hen corpuscles, labelled by their active desoxyribose nucleic acid content, can be used in two different ways. We can administer, for

⁽¹⁾ HAHN, P. F., BALE, W. F., ROSS, J. F., HETTIG, R. A., and WHIPPLE, G. H., *Science*, **92**, 131 (1940).

⁽²⁾ HAHN, P. F., BALE W., F., and BALFOUR, W. M., *Amer. J. Physiol.*, **135**, 800 (1941--42).

example, labelled phosphate to the hen, and after the lapse of a week replace part of the corpuscles of a second hen by labelled corpuscles of the first one. When taking blood samples at intervals, we can determine what percentage of the transfused corpuscles is still present in the circulation of the hen. In a note to be published later, we shall communicate the results obtained in such experiments. In this note we shall describe another method in which, by avoidance of blood transfusion, the uncertainty about the equality of the life-time of the transfused corpuscles and the endogenous corpuscles can be eliminated.



Life-cycle of the red corpuscles of two hens. Abscissae: days after start of experiment; ordinates: specific activity of desoxyribose-nucleic acid phosphorus extracted from the corpuscles secured at different dates.

In the latter method, labelled phosphate is administered twice a day to the hen in such quantities that the plasma phosphate is kept at a constant or almost constant level of activity. The active phosphate penetrates into the marrow and participates in the formation of the nucleic acid of the corpuscles, which thus become labelled. The percentage of labelled corpuscles will increase with time, and finally the circulation will contain labelled corpuscles only; thus the activity of 1 mgm corpuscle desoxyribose nucleic acid phosphorus will be equal to the activity of 1 mgm marrow phosphorus and 1 mgm plasma phosphorus respectively.

The results of such experiments are shown in the accompanying graph, which makes it clear that in the first four days the nucleic acid present in the corpuscles is inactive. This may be interpreted by assuming that,

in the first phase of the experiment, corpuscles containing inactive nucleic acid reach the circulation, and that it is about four days before corpuscles containing labelled nucleic acid are given off by the sinusoids to the circulation. The maturing of the corpuscles in the marrow thus takes about four days. The graph also shows that, after the lapse of about thirty-three days, the maximum value of the activity of the desoxyribose nucleic acid is reached. Taking into account that in the first four days no labelled corpuscles intrude into the circulation, the life-time of the red corpuscles will be 29 days. It is of interest finally to note that the results obtained indicate that all or almost all corpuscles present in the circulation have a similar life-time.

COMMENT ON PAPERS 67—70.

WE started our investigations on the application of radio-phosphorus as a tracer with the study of the incorporation of ^{32}P into the mineral constituents of the skeleton, then took up the study of the formation of labelled phosphatides and acid-soluble compounds. In 1939 an investigation of incorporation of ^{32}P into desoxyribonucleic acid was started. A note on the results of these studies was published by HAHN and HEVESY (1940) and a more detailed account is given in paper 67. Outgrown organs which do not secrete DNA containing leucocytes as the liver and kidney were found to incorporate minimal amounts of ^{32}P into DNA only. The spleen which secretes leucocytes incorporates appreciable amounts of ^{32}P into its DNA since the secreted molecules have to be replaced and the new formation of DNA molecules takes place under the incorporation of ^{32}P . The production of labelled DNA molecules is very pronounced in the intestinal mucosa in which the cells destroyed in the course of the digestive processes have to be replaced. In paper 67 it is stated that "The rate of renewal of the nucleic acid in the liver may be identical with the rate of formation of liver cells". A statement, supported by the results of recent investigations.

In later studies (paper 68) the rate of formation of both labelled DNA and RNA was investigated. In the meantime BRUES *et al.* (1944) found, in experiments taking over a week, the latter to be larger than the former. The very great difference between the incorporation of ^{32}P into DNA and the RNA of the rat liver was brought out in the investigation described in paper 68 in which incorporation was determined after 2 hr only. The specific activity of RNA phosphorus was found to be thirty-three times as large as that of the DNA phosphorus. In the spleen and intestine in which DNA is built up at a rapid rate the corresponding ratio makes out 3 and 2 only. In the rapidly growing Jensen-sarcoma of the rat in which formation of DNA takes place at a very appreciable rate as it does in the spleen and in the intestinal mucosa, the corresponding ratio was found to be 2.4 (paper 69). The great stability of the DNA present in nucleated erythrocytes made it possible to determine the previously unknown life cycle of avian red corpuscles. Doubts were expressed on the correctness of the results obtained when these were communicated at the Solvay Congress in Brussels. Their correctness was, however, brought out by later investigations (SHEMIN, 1948; OTTESEN, 1955). Since those early days when ^{32}P was first incorporated into DNA, labelled desoxyribonucleic acid found a very extended application in numerous studies.

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71. THE EFFECT OF X-RAYS ON THE RATE OF NUCLEIC ACID FORMATION IN JENSEN-SARCOMA

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THE detection of the effect of X-rays on sarcomas is usually studied in the following way. Fragments of the irradiated sarcoma are transferred by inoculating normal animals and tests are made to find out whether or not the sarcoma has grown after a few days. We have tried to replace this procedure by a chemical test. There are numerous experiences which support the statement that doses which are effective against sarcoma do not substantially affect the metabolic processes taking place in the tissue cells, and that are the processes taking place in the cell nucleus which primarily succumb to the action of the radiation. In the search for a chemical test for the effect of X-rays on the sarcoma it was therefore evident that the processes occurring in the cell nucleus should be studied in more detail. The nucleic acids are among the most important constituents of the cell nucleus.

Nucleic acid plays an essential part in cell division. KOSSEL, for example, has shown that the changes occurring in the course of spermatogenesis consist in degradation and synthesis of proteins and in the synthesis of the histonamine or protamine nucleate, which is comparatively poor in protein and CASPERSSON's⁽¹⁾ studies of the absorption of ultra-violet radiation by dormant and dividing cell nuclei have made it apparent that KOSSEL's scheme of protein synthesis is valid also for the ordinary mitotic cell division.

The mitotic division of cells is retarded by comparatively small doses of X-rays and, on the basis of the above discussion, it might be expected that the irradiation of tissue with X-rays would cause a diminution in the of rate of formation of desoxyribo nucleic acid in the cell nucleus. This view has induced us to study the formation of desoxyribo nucleic acid in the Jensen-sarcoma of the rat, before and after irradiation with X-rays.

¹ T. CASPERSSON, *Chromosoma* **1**, 562 (1940).

The investigation was performed by using the radioactive indicator method. This method makes it possible to distinguish between the molecules of nucleic acid which have been formed before and after the beginning of the experiment. The latter, which have been formed in an active medium, viz. in cells containing active phosphate, will be active (i. e. will contain radioactive phosphorus) as opposed to the inactive molecules already present before commencement of the experiment.

DESCRIPTION OF THE METHOD

When a very small amount of sodium phosphate, labelled with an admixture of radioactive phosphorus ($^{32}_{15}\text{P}$), is introduced by, for example, injection into the experimental animal, the labelled phosphate ions soon enter the sarcoma cells and take part in the synthetic processes occurring in these cells with the same probability as the other phosphate ions already present in the sarcoma cells. If molecules of nucleic acid are synthesized in the sarcoma cells they will be radioactively labelled. If all the nucleic acid molecules present in the sarcoma at the end of the experiment have been formed in the course of the experiment, then 1 mgm of nucleic acid phosphorus will show the same content of ^{32}P , i. e. the same radioactivity, as 1 mgm of phosphate phosphorus. If, on the other hand, at the conclusion of the experiment 1 mgm of nucleic acid phosphorus shows an activity amounting to, say, only 1 per cent of the activity of 1 mg of phosphate phosphorus, then the amount of nucleic acid formed during the experiment will be 1 per cent of the total amount of nucleic acid present in the sarcoma. By making this statement we assume that the measured activity of 1 mgm of phosphate after completion of the experiment is equal to the activity present at any other point in time during the course of the experiment (cf. the discussions on p. 705).

Preparation and Measurement of the Activity of Radioactive Phosphorus

The radioactive phosphorus used in the experiments which will be described below has usually been obtained by the action of neutrons on carbon disulphide¹. A mixture of radium-beryllium containing 600 mgm of elementary radium served as the source of neutrons. We are very greatly indebted to Professor NIELS BOHR for the loan of this source and also for many other pieces of equipment. In addition to this, radioactive phosphorus was available which had also been produced by the

¹ O. CHIEVITZ and G. HEVESY, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **13**, 9 (1937).

action of fast neutrons on carbon disulphide but in this case the neutrons had been obtained by means of a high voltage apparatus, usually at the Institute of Theoretical Physics of the University of Copenhagen but occasionally at the Research Laboratory of Philips Gloeilampfabrik in Eindhoven.

The samples produced with the help of the radium sources did not contain any chemically detectable amount of phosphorus. They were obtained by shaking the 10 l. of carbon disulphide used for irradiation with dilute nitric acid. The active phosphate remained in the residue after evaporating the nitric acid solution; it was taken up in water and the solution was filtered through a glass filter. This process was repeated a few times. The activity was finally dissolved in physiological saline solution and then injected into the experimental animals; 0.1—1.0 cm³ was injected into each rat. The activity of the injected solution amounted to about 0.1 μ c.

Measurement of the Radioactivity of the Nucleic Acid Phosphorus

The rat was killed 2 hr after injection and the nucleic acid of the sarcoma was isolated. The KLEIN and BECK² method of isolation was used for this purpose. Besides the weakly active nucleic acid, very strongly active acid-soluble compounds and strongly active phosphatides are also formed in the sarcoma in the course of the experiment; contamination of the nucleic acid with the slightest amount of acid-soluble phosphorus or with phosphatide phosphorus can therefore interfere with the results of measurement of the nucleic acid activity. For this reason, the samples of nucleic acid were purified still more thoroughly than in the above-mentioned experiments of KLEIN and BECK. The purified nucleic acid was wet-ashed with sulphuric acid and 30% H₂O₂. One-fifth of the solution obtained was used for the colorimetric determination of phosphorus; four-fifths of the solution were treated with 80 mgm of sodium phosphate and the phosphorus content was isolated as magnesium ammonium phosphate. By this means the active phosphorus content of the sample was present in a mixture with about 70 mgm of inactive magnesium ammonium phosphate. All our preparations were treated in this way and we were thus able, in determining the activity, always to compare samples of nearly the same weights and volumes. Any contingent correction for different absorption of β -radiation in the samples being compared, was thus avoided.

The activity of the magnesium ammonium phosphate or of a known fraction of the sample was measured with a Geiger—Müller counter. The activity of 1 mgm of phosphorus was calculated from the measured

² O. KLEIN and BECK, *Z. Krebsforsch.* **42**, 172 (1935).

activity, the weight of the magnesium ammonium phosphate sample and the chemically determined phosphorus content of the nucleic acid as shown in the following example:

The activity of 1 mgm of nucleic acid phosphorus is equal to $8.60 \times 80 \times 5 / 2.44 \times 71 \times 4 = 4.98$ counting-tube pulses/min. The figure 8.60 denotes the activity of the magnesium ammonium phosphate containing the nucleic acid phosphorus, 2.44 is the phosphorus content of the total nucleic acid in the sarcoma (mgm); 80 is the weight of the whole of the precipitated magnesium ammonium phosphate sample (mgm), and 71 is the weight of sample placed under the counting tube; $5/4$ is the correction to be applied because only four-fifths of the solution obtained by ashing the nucleic acid was used for the radioactive investigation.

After this it is necessary to compare the radioactivity of 1 mgm of nucleic acid phosphorus with the radioactivity of 1 mgm of free-phosphate phosphorus in the sarcoma. The postulate mentioned on p. 690, that the phosphate phosphorus isolated from the sarcoma has the same activity which would have been found at any time during the experiment, does not hold good. The absorption of the injected phosphate requires several minutes and although the membrane of sarcoma cells is, as discussed elsewhere⁽¹⁾, highly permeable to the penetration of phosphate, the infiltration of phosphate from the plasma (or from the extracellular fluid) into the sarcoma cells likewise requires time. Furthermore, the activity of the plasma phosphate changes during the experiment. At first it increases and then decreases, as a result of the inflow of labelled phosphate into the cells of the organ and vice versa. Strictly speaking, therefore, we should measure the activity of 1 mgm of sarcoma phosphate at different times and in this way calculate the mean value of the phosphate activity during the experiment. This average activity of 1 mgm of phosphate should then be compared with the activity of 1 mgm of nucleic acid phosphorus determined at the conclusion of the experiment. We are, however, not so much interested in the accurate values of nucleic acid metabolism of this acid in the sarcoma as in the effect of X-rays on the nucleic acid metabolism. We have, therefore, replaced the above somewhat tedious procedure by the following one.

The activity of 1 mgm of nucleic acid phosphorus was compared with the activity of 1 mgm of free plasma phosphorus determined at the end of the experiment. The activity of the plasma phosphorus at first increases during the experiment and later decreases; the activity value measured two hours after injection is not very different from the mean value in the course of the experiment. We have also compared the activity of 1 mgm of nucleic acid of the sarcoma with the activity of 1 mgm of free phosphorus in the liver, and have thus attained a second scale for com-

¹G. HEVESY and H. EULER, *Ark. Kem* 15A, 15 (1940).

parison. In our later experiments we have also compared the activity of the nucleic acid with that of the free phosphorus in the sarcoma. In the latter procedure a portion of the sarcoma must be sacrificed in order to isolate the free phosphate; in spite of this the procedure is definitely preferred to the one first described and at first applied in our experiments.

Plasma and liver samples were treated with 10 and 25% trichloroacetic acid, respectively; 80 mgm of sodium phosphate was added to four-fifths of the solution, the free phosphate in the solution was precipitated as magnesium ammonium phosphate and the radioactivity of the sample was measured, as described above, with the Geiger—Müller counter. One-fifth of the solution was used for colorimetric determination of the free phosphorus. The magnesium ammonium phosphate samples were placed in aluminium trays, 1.2 cm in diameter and 2 mm deep, before the activity was measured; the trays were pushed beneath the window of the counter. Whereas the liver fractions measured yielded more than 1000 pulses/min in the counting tube, the activity due to the nucleic acid of the sarcoma usually amounted to only a few pulses per minute; in isolated cases, indeed, it was necessary to measure activities amounting to only a few-tenths of a pulse per minute. Such measurements were performed by alternating measurements of the activity of the sample and of inactive magnesium ammonium phosphate for periods of 24 hr each. The background amounted to about 4 pulses/min and could be reproduced with an accuracy of 2 per cent. Temperature variations of the counting apparatus in excess of about 1° C must be avoided if such accuracy is to be attained.

Determination of the Percentage of Injected ^{32}P Found in the Nucleic Acid of the Sarcoma

In determining the percentage of the injected radioactively labelled phosphorus incorporated in the nucleic acid of the sarcoma, the procedure is as follows: 80 mgm of sodium phosphate are added to a known small fraction, e. g. one-threehundredth of the solution used for injection; the P content of the solution is then precipitated as magnesium ammonium phosphate. The activity of this sample is compared with the activity of the nucleic acid phosphorus, isolated from the sarcoma, which is also in the form of magnesium ammonium phosphate and of the same weight as the above sample. If the activity of one-threehundredth of the injected solution is equal to 100 pulses/min, then 30,000 pulses were injected into the rat. If the activity of 1 mgm of nucleic acid phosphorus is found to be 10, then 1 mgm of nucleic acid phosphorus contains 0.033 per cent of the injected ^{32}P . The values recorded in Tables 1 to

6 are obtained by dividing by the colorimetrically determined weight of the phosphorus in the nucleic acid.

The Sarcomas Used

Jensen sarcomas of rats, which had been cultivated by transplanting a sarcoma obtained from Professor DOMAGK were used for all the experiments which we have described up to this point.

The sarcomas were always transplanted subcutaneously by installing a tissue section about 1 mm thick. The sarcomas developed in our strain of rats up to a size of about 20 gm in about 3 weeks. Rats with a sarcoma weight of about 20–30 gm were ordinarily used in the irradiation experiments.

Isolation of the Nucleic Acid

The sarcoma tissue was finely minced and worked up for desoxyribo nucleic acid by the method described by KLEIN and BECK⁽¹⁾. The crude precipitate was dissolved in 1 N caustic soda, treated with about 80 mgm of disodium hydrogen phosphate and precipitated with 5% ferric hydroxide solution. This purification of the nucleic acid was repeated two more times to remove any radioactive phosphate. The product obtained in this way was then twice dissolved and reprecipitated, with methyl alcohol containing hydrochloric acid, in accordance with the method of KLEIN and BECK. The most careful purification of the nucleic acid from foreign phosphates is of the utmost importance because the acid-soluble phosphate is much more active than the phosphate from the nucleic acid.

The phosphate in the finally purified precipitate was determined colorimetrically by the method of FISKE and SUBBAROW⁽²⁾ (THEORELL's modification⁽³⁾). Another portion was precipitated as magnesium ammonium phosphate which was then used for determining the activity.

With regard to the quantities employed in this procedure, the reader is referred to the description supplied as an example (p. 692) and also to Tables 1–6 (pp. 699–702).

The samples of blood and liver were worked up by the methods described below:

The blood, which had been collected in a vessel containing a few milligrams of sodium citrate, was centrifuged; the plasma (usually 2–3 cm³) was treated with 3 cm³ of 10% trichloroacetic acid, the

¹ O. KLEIN and BECK, *Z. Krebsforsch.* **42**, 163 (1935).

² FISKE and SUBBAROW, *J. Biol. Chem.* **66**, 375 (1925).

³ THEORELL, *Biochem. Z.* **230**, 1 (1931).

solution centrifuged and filtered, and the centrifuged residue was again extracted with 2 cm³ of trichloroacetic acid and filtered. The whole of the filtrate was diluted with water to 25 cm³; 2 cm³ of this was used for colorimetric determination of the phosphorus by the Fiske—Subbarow—Theorell method; 20 cm³ of the filtrate was treated with 80 mgm of disodium hydrogen phosphate, and the whole of the phosphate was precipitated as magnesium ammonium phosphate. After drying at 110° C this precipitate was used for determination of the activity.

The liver (usually 5 to 8 gm) was first treated for 15 min with 20 cm³ of 25% trichloroacetic acid and for a second period of 10 min. The trichloroacetic acid extract was filtered and the filtrate diluted with water to 100 cm³. Phosphorus was determined colorimetrically in 1 cm³. After addition of 80 mgm of sodium phosphate, 80 cm³ of the solution was used for precipitation of the magnesium ammonium phosphate required for the determination of the radioactivity.

Irradiation of the Sarcoma

The sarcomas were irradiated for periods of from 26—67 min with X-rays emitted from a tube operated at 165 kV and 7 mA. A 0.5 mm copper foil and 1 mm aluminium foil were used as radiation filters. The irradiation was made at distances between 25 and 42 cm. Only the sarcoma was irradiated; the other parts of the body of the rat were protected against the action of the radiation by covering them with lead plates. The NaCl solution containing the radioactively labelled sodium phosphate was injected 20 min after completion of the irradiation.

The performance of experiments lasting for such a short time has the advantage, among others, that the effect of the X-rays can be studied soon after the ending of the irradiation. It is well known that in the course of time considerable changes take place in irradiated tissue and the effect of these changes on the nucleic acid metabolism can be studied by injecting the radioactive one or more days and not immediately after interruption of the irradiation. The results of such experiments will be discussed below.

EXPERIMENTAL RESULTS

The results of the experimental work can be seen in Tables 1—6. Tables 1—3 contain data on the rate of formation of desoxyribo nucleic acid in unirradiated and in slightly and more strongly irradiated sarcomas. Tables 4—6 contain data on the fraction of injected ³²P which is to be found after the passing of 2 hr in 1 mgm of free plasma phosphate phosphorus and in 1 mgm of free liver phosphate phosphorus.

TABLE 1. — NUCLEIC ACID FORMATION IN UNIRRADIATED JENSEN-SARCOMA
(Time of experiment, 2 hr.)

Rat no.	Weight of purified sarcoma (gm)	Nucleic acid phosphorus content in 1 gm sarcoma (mgm)	³² P content of 1 mgm nucleic acid phosphorus, as a percentage of the ³² P content of	
			1 mgm of inorg. P of the liver	1 mgm of inorg. P of the plasma
I + II	36.2	3.8	2.60	—
III + IV	34.5	12.3	2.23	—
V + VI	19.3	7.5	3.10	—
VII + VIII	31.9	6.6	1.76	2.98
IX + X	23.2	8.3	1.30	2.15
XI + XII	2.9	0.9	1.35	—
XIII	16.7	1.3	2.33	1.76
XIV	22.5	6.3	1.14	1.06
XV	17.9	2.4	1.13	1.21
XVI	17.7	5.7	1.25	1.52
Mean value	22.3	5.5	1.82	1.78

TABLE 2. — NUCLEIC ACID FORMATION IN SLIGHTLY IRRADIATED JENSEN-SARCOMA
(Dosage of 77 to 310 r [international Roentgen units]. Time of experiment, 2 hr.)

Marking of rat	Dosage (r)	Weight of purified sarcoma (gm)	Nucleic acid P content of sarcoma (gm)	³² P content of 1 mgm nucleic acid P as a percentage of the ³² P content of	
				1 mgm of inorg. P of the liver	1 mgm of inorg. P of the plasma
A	77	6.2	10.1	1.74	1.53
B	77	11.2	16.0	0.26	0.22
C	77	5.7	11.0	1.33	1.74
D	155	13.0	6.4	1.39	1.03
E	155	9.4	13.7	1.80	3.41
F	155	15.0	35.0	1.92	2.54
G	310	13.8	1.3	1.20	1.47
H	92	12.3	2.3	0.95	0.80
J	92	10.0	1.0	1.04	1.53
K	92	3.1	2.5	1.65	1.51
L	186	6.3	5.0	2.14	3.32
M	186	6.8	1.2	1.93	3.71
N	186	3.8	0.47	0.54	0.80
Mean value	—	9.0	8.1	1.38	1.82

TABLE 3. — NUCLEIC ACID FORMATION IN IRRADIATED JENSEN-SARCOMA
(Dosage of 460 to 7000 r [international Roentgen units]). Time of experiment 2 hr)

Rat no.	Dosage (r)	Weight of purified sarcoma (gm)	Nucleic acid P content of sarcoma (gm)	³² P content of 1 mgm nucleic acid P as a percentage of the ³² P content of	
				1 mgm of inorg. P of the liver	1 mgm of inorg. P of the plasma
1	2080	11.1	1.33	0.20	—
2	2080	18.2	2.33	0.41	—
3	2080	18.5	8.30	0.37	—
4	2080	5.3	1.36	0.17	0.32
5	2080	5.5	2.30	0.20	0.27
6	2080	6.0	1.86	0.21	0.44
7	1000	14.9	4.05	0.22	—
8	1000	13.0	2.05	0.35	—
9	1000	6.8	1.70	0.39	—
10	2080	18.0	0.36	0.10	0.19
11	1240	13.4	1.33	0.25	0.48
12	620	17.0	2.11	0.13	0.24
13	1025	13.9	6.20	0.36	0.21
14	460	12.0	5.30	0.02	0.01
15	460	8.6	5.31	0.56	0.29
16	1025	5.9	21.0	0.28	0.35
17	1025	2.2	6.71	1.04	1.50
18	1025	11.8	52.0	0.31	0.54
19	1025	3.4	6.70	0.51	0.66
20	465	9.7	16.2	0.84	1.04
21	465	10.0	27.2	2.12	1.29
22	620	6.3	8.80	1.31	3.41
23	900	7.7	25.0	0.78	0.99
24	1180	4.8	2.31	0.60	0.82
25	1395	4.6	2.00	0.42	0.56
26	1025	7.3	7.12	0.80	0.91
27	1025	17.5	10.9	0.076	0.18
28	1025	6.7	11.0	0.65	0.70
29	1180	12.3	4.72	0.34	0.34
30	1400	6.0	3.70	0.47	0.64
31	1730	9.3	2.27	1.40	1.51
32	2550	8.2	1.80	0.75	0.95
33	7000	9.1	0.28	0.40	0.50
34	7000	14.2	0.65	0.11	0.080
Mean value	—	9.9	7.5	0.50	0.65

TABLE 4. — ^{32}P CONTENT OF THE FREE PHOSPHATE IN THE BLOOD PLASMA AND LIVER
(Unirradiated)

Rat no.	Weight of liver (gm)	P content of liver phosphate (mgm%)	P content of plasma phosphate (mgm%)	% of injected ^{32}P present in	
				1 mgm liver P	1 mgm plasma P
I + II	16.9	39	—	0.48	—
III + IV	17.0	50	—	0.44	—
V + VI	15.2	52	—	0.48	—
VII + VIII	13.6	57	5.7	0.85	0.59
IX + X	14.5	58	6.4	0.89	0.61
XI + XII	12.2	45	—	1.37	—
XIII	6.0	53	8.2	1.35	1.79
XIV	5.0	58	8.5	1.52	1.61
XV	6.3	47	7.7	1.42	1.32
XVI	6.4	46	9.6	1.63	1.33
Mean value	7.1	51	7.7	1.04	1.21

TABLE 5. — ^{32}P CONTENT OF THE FREE PHOSPHATE IN THE BLOOD PLASMA AND LIVER
(Slightly irradiated, 77—310 r.)

Marking of rat	Weight of liver (gm)	P content of liver phosphate (mgm%)	P content of plasma phosphate (mgm%)	% of injected ^{32}P present in	
				1 mgm liver P	1 mgm plasma P
A	6.6	49	4.0	2.27	2.60
B	6.8	54	4.3	2.31	2.74
C	6.5	53	4.4	2.84	2.75
D	6.2	54	4.0	2.19	2.97
E	7.0	41	7.8	2.31	1.08
F	5.9	54	5.6	2.23	1.64
G	6.6	46	9.2	1.47	1.21
H	8.3	50	6.4	1.55	1.84
J	6.6	48	6.5	2.23	1.52
K	5.9	42	5.6	1.88	2.05
L	5.7	43	7.4	1.89	1.22
M	6.0	40	7.9	1.92	1.00
N	5.2	53	6.0	1.59	1.81
Mean value	6.3	47	6.1	2.05	1.83

TABLE 6. — ^{32}P CONTENT OF THE FREE PHOSPHATE IN THE BLOOD PLASMA AND LIVER
(Irradiated, dosage 460—7000 r.)

Rat no.	Weight of liver (gm)	P content of liver phosphate (mgm%)	P content of plasma phosphate (mgm%)	% of injected ^{32}P present in	
				1 mgm liver P	1 mgm plasma P
1	4.8	79	—	0.81	—
2	5.2	69	—	0.88	—
3	8.0	52	—	0.79	—
4	5.9	50	7.4	1.76	0.94
5	7.1	55	6.8	1.25	0.91
6	4.0	48	6.8	3.15	1.51
7	7.0	47	—	0.67	—
8	6.6	36	—	0.43	—
9	5.2	41	—	0.37	—
10	4.4	48	10.0	4.55	2.31
11	5.0	45	6.9	4.45	2.33
12	4.6	48	8.3	3.90	2.24
13	5.5	79	5.7	1.94	3.50
14	5.1	89	4.5	1.70	3.21
15	4.1	98	6.0	1.77	3.23
16	5.9	49	9.2	3.0	2.5
17	7.2	45	5.6	2.5	2.1
18	4.0	53	4.4	4.7	2.7
19	5.8	52	4.9	3.6	2.7
20	6.2	60	7.9	1.5	1.0
21	6.1	64	7.7	1.1	1.8
22	6.6	46	9.2	1.7	0.6
23	4.8	69	7.6	1.8	1.3
24	5.5	60	5.9	1.7	1.1
25	4.9	67	4.0	1.7	1.7
26	7.3	50	6.6	1.2	1.1
27	7.5	59	6.7	1.8	0.9
28	6.7	57	5.0	1.3	0.7
29	5.1	27	5.4	3.6	1.5
30	5.4	46	5.0	1.9	1.4
31	6.5	54	4.5	0.9	0.9
32	6.2	52	5.8	1.3	1.0
33	5.3	58	5.8	1.7	1.4
34	5.5	62	9.6	1.5	2.1
Mean value	5.7	59	6.0	2.0	1.7

DISCUSSION OF RESULTS

The data in Tables 1 to 3 and the summarizing presentation in Table 7 show that the formation of new (radioactive) molecules of desoxyribo nucleic acid is in most cases markedly reduced by the action of an X-ray dosage exceeding 450 r. In sarcomas irradiated with less than 450 r

the effect of irradiation on the formation of radioactivelylabelled nucleic acid is less pronounced.

In five of the thirty-four sarcomas irradiated with more than 450 r we were unable to detect any effect of irradiation on the formation of

TABLE 7. — NUCLEIC ACID METABOLISM IN UNIRRADIATED, SLIGHTLY IRRADIATED (WITH LESS THAN 450 r) AND MORE STRONGLY IRRADIATED (MORE THAN 450 r) JENSEN SARCOMA

Sarcoma	³² P content of 1 mgm nucleic acid P as a percentage of the P ³² content of	
	1 mgm inorg. liver P	1 mgm inorg. plasma P
	Mean value	
Unirradiated	1.82	1.78
Slightly irradiated	1.38	1.81
Irradiated	1.50	0.65
	Maximum value	
Unirradiated	3.10	2.98
Slightly irradiated	2.14	3.11
Irradiated	2.12	3.41
	Minimum value	
Unirradiated	1.13	1.06
Slightly irradiated	0.26	0.22
Irradiated	0.076	0.080

nucleic acid. One of these sarcomas was irradiated with 1730 r, a second with 1025 r, a third with 620 r and the remaining two with 465. Presumably these sarcomas were more resistant to the action of radiation. In the investigation of the growth of irradiated sarcomas after transplantation, it was found that the individual sarcomas vary in sensitivity toward the action of radiation. Russ and Scott⁽¹⁾, for example, state that of one hundred Jensen rat sarcomas irradiated with 1000 r, seventy-five recovered, and SUGURIA² states, with regard to the sensitivity of the closely related "sarcoma 180", that only half of the sarcomas withered after irradiation with 1000 r.

In most cases the difference in the formation of radioactively labelled (and thus newly formed) nucleic acid molecules in unirradiated and irradiated sarcomas is so striking that an unirradiated sarcoma can be distinguished from an irradiated one by just placing the sample under the counting tube. Complete suppression of the formation of active nucleic acid molecules cannot of course be achieved even by using very powerful doses. For example, after the most effective irradiation with

¹ S. RUSS and G. M. SCOTT, *Brit. J. Radiol.* **13**, 267 (1940).

² K. SUGURIA, *Radiology* **29**, 352 (1937).

7000 r the activity of 1 mgm of nucleic acid phosphorus was found to be equal to 0.1 per cent of the activity of 1 mgm of free liver phosphorus and to 0.08 per cent of the activity of 1 mgm of free plasma phosphorus. The experiments performed up to the present time with Ehrlich carcinomas on mice have to some extent produced results differing from those obtained with Jensen sarcomas, as will be shown in a future communication.

By means of the possibility of measuring the effect exerted by X-rays on the formation of nucleic acid molecules in a sarcoma with the aid of radioactive indicators, their action on the sarcomas can be followed chemically. It should be noted that the experiments described are easily performed, preferably by comparing the activities of the nucleic acid phosphorus in the sarcoma and of the free phosphorus in the sarcoma, and also that it would be important to establish the effective dose of X-rays causing a detectable reduction on the formation of radioactively labelled nucleic acid.

In the experiments described, the formation of radioactively labelled nucleic acid was determined during the 2 hr following irradiation (the irradiation itself lasted for at most 42 min). There is nothing to hinder determination of the formation of radioactively labelled nucleic acid in the first hour, the first half-hour or even shorter times after the irradiation. It is thus possible to determine the chemical effect of X-rays on the sarcoma immediately after irradiation.

Phosphorus Content of Liver and Plasma

The data in Tables 4—6 show that there is no essential difference between the phosphorus contents of the plasma and liver in unirradiated, slightly irradiated and more strongly irradiated animals (in all cases only the sarcoma was irradiated). The average free phosphorus content of the liver amounts to 51, 47 and 59 mgm %; the corresponding values for the free phosphorus content in the plasma are 7.7, 6.1 and 6.0 mgm %.

Two hours after injection there is 1.04⁽¹⁾, 2.05 and 2.00 per cent of the injected ³²P in 1 mgm of free liver phosphorus in unirradiated, slightly irradiated and more strongly irradiated rats, respectively. The liver therefore contains at this time (mean value of liver weight equals 7.1, 6.3 and 5.7 gm) about 7 per cent of the injected ³²P as free P.

In 1 mgm of free plasma phosphorus 2 hr after injection there is 1.21, 1.83 and 1.72 per cent of the injected ³²P. Therefore 1 mgm of free liver

¹In later experiments (see p. 706) 2.13 and 2.16 per cent of the injected ³²P was found, respectively, in 1 mgm of free liver phosphorus in unirradiated rats and in animals irradiated with 2000 r. The corresponding values for the plasma phosphorus were 1.21 and 1.15.

phosphorus contains nearly the same amount of ^{32}P as 1 mgm of free plasma phosphorus 2 hr after injection.

Comparison of the Activity of Nucleic Acid Phosphorus with the Activity of the Free Phosphorus in the Sarcoma

It is evident from the preceding section that there is less active nucleic acid formed in irradiated than in unirradiated sarcoma. It cannot be concluded unconditionally from this statement, however, that the irradiation decreases the rate of formation of nucleic acid in the sarcoma. It might be thought that irradiation would make it difficult for the radioactive tracer to penetrate into the sarcoma cells and that the observed would be due to a decreased permeability of the cell wall to phosphate under the action of the irradiation. The experiences which have been accumulated from various biological systems concerning the action of irradiation on permeability, and which will be reported shortly, are opposed to the latter interpretation of the observed decreased formation of active nucleic acid in the irradiated sarcoma; we have, however, set up experiments, in which the activity of the nucleic acid phosphorus was compared with that of the free phosphorus in the sarcoma, in order to eliminate the explanation by the effect of irradiation on the phosphate permeability. The major portion of the free phosphorus in the sarcoma consists of phosphorus situated within the cells; if a comparison of the ratio of the activities of the nucleic acid P and the free P of the sarcoma indicates a considerably smaller value in the case of the irradiated sarcoma, then this finding proves unequivocally that irradiation inhibits the metabolism of nucleic acid¹.

The results of these experiments are seen in Tables 8 and 9. These tables show that the radioactively labelled nucleic acid, i.e. the nucleic acid formed during the 2 hr experimental period after irradiation with 2000 r, was about one-third the amount formed in the unirradiated sarcoma. This result is supported by data obtained by a comparison of the activities of nucleic acid phosphorus and free sarcoma phosphorus in the fresh tissue material. (The behaviour of the necrotic tissue will be discussed in the next section.)

As has already been mentioned, the free phosphorus of the sarcoma consists partly of phosphorus originating in the extracellular fluid of the tissue, and this phosphorus has a specific activity (activity per mgm P) which differs from that of the intracellular P. The specific activity of

¹ In our earlier experiments we have omitted a study of the activity of free phosphorus in the sarcoma to enable us to use all the sarcoma tissue in obtaining nucleic acid; later, however, we found it decidedly advisable to determine also the specific activity of the free phosphorus in every sarcoma under investigation.

the extracellular P corresponds closely to the activity of the free plasma phosphorus. The extracellular share of the free sarcoma phosphorus, however, is small and, since the activity of the sarcoma phosphorus does not differ considerably from that of the plasma phosphorus, the error committed in disregarding the complex nature of the free sarcoma phosphorus is not important. The rat 47 : 1, for example, contains 1.45 mgm % of extracellular P and 44.8 mgm % of intracellular P. These figures are obtained by assuming that one-quarter of the sarcoma consists of extracellular fluid in conjunction with the P content of the plasma (5.8 mgm %) and of the sarcoma (46.3 mgm %). The specific activity of the 1.45 mgm % of extracellular P is 5 per cent higher than that of the 44.8 mgm % of intracellular P (see Table 8); by neglecting the extracellular quota, therefore, the specific activity of the intracellular P is overestimated by $0.07/44.8 = 0.14$ per cent.

With regard to the sarcoma cells there is possibly a limited effect of irradiation on their permeability. Whereas the ratio of the activity of 1 mgm of sarcoma P to that of 1 mgm of plasma P in unirradiated sarcomas is on the average 1.07 (see Tables 8 and 9), the corresponding ratio in irradiated sarcomas is 0.94; this difference, however, cannot explain the major part of the observed effect.

Nucleic Acid Metabolism and Phosphate Permeability of the Necrotic Sarcoma Tissue

It is shown in Table 8 that the nucleic acid formation in necrotic sarcoma tissue is indeed considerably less than in fresh tissue but that

TABLE 8. — NUCLEIC ACID FORMATION IN UNIRRADIATED SARCOMAS

R a t	Volume of sarcoma (cm ³)	Activity of 1 mgm of nucleic acid phosphorus as a percentage of the activity in 1 mgm of			
		liver P	plasma P	sarcoma P	
47 : 1 (200 gm)	38	{ fresh necrotic	0.77 0.41	1.13 0.55	1.18 1.08
47 : 2 (145 gm)	13	{ fresh necrotic	1.58 —	3.31 —	2.12 —
47 : 3 (158 gm)	14	{ fresh necrotic	1.15 0.71	1.67 1.03	1.23 1.20
47 : 4 (168 gm)	18	{ fresh necrotic	0.81 0.29	2.77 0.97	1.93 1.15
51 : 1 (160 gm)	19 + 15	{ fresh	0.97	1.23	4.64
+ 51 : 2 (132 gm)		{ necrotic	0.78	0.96	1.06
48 : 4b (188 gm)	23		1.13	1.47	1.20
Mean value		{ fresh	1.07	1.93	2.05
		{ necrotic	0.55	0.88	1.12

the former is not negligible. The same is true of the speed of replacement of intracellular phosphate by plasma (lymphatic) phosphate. The exchange equilibrium in necrotic tissue has not proceeded as far as in the fresh tissue; in the course of 2 hr, however, a considerable portion of the free sarcoma phosphate is replaced by plasma phosphate. Hence it follows that a good circulation of blood (lymph) must exist in part of the necrotic tissue. The intrinsically decreased rate of formation of nucleic acid in necrotic tissue is reduced still further by the action of the X-rays.

Percentage of injected ^{32}P present in 1 mgm

	Liver P	Plasma P	Sarcoma P		Nucleic acid P	
			fresh	necrotic	fresh	necrotic
47 : 1	1.73	1.18	1.12	0.635	0.0133	0.0065
47 : 2	1.99	0.95	1.49	1.29	0.0313	—
47 : 3	1.83	1.26	1.72	1.08	0.0212	0.0130
47 : 4	3.73	1.12	1.61	0.95	0.0311	0.0109
51 : +2	1.98	1.56	0.46	1.45	0.019	0.015
48 : 4b	1.51	1.21	1.43	—	0.017	—
Mean value	2.13	1.21	1.30	1.08	0.022	0.011

TABLE 9. — NUCLEIC ACID SYNTHESIS IN SARCOMAS IRRADIATED AT 80 r/min FOR 25 min

Rat	Volume of sarcoma (cm ³)	Activity of 1 mgm nucleic acid phosphorus as a percentage of the activity in 1 mgm of			
		liver P	plasma P	sarcoma P	
49 : 1	18	{ fresh necrotic	0.68 0.40	0.90 0.60	0.72 0.88
49 : 2	23	{ fresh necrotic	1.00 0.42	0.85 0.33	0.79 0.46
49 : 3	13	{ fresh necrotic	0.70 0.025	1.01 0.036	1.00 0.036
49 : 4	19	{ fresh necrotic	0.12 —	0.50 —	0.54 —
50 : 1	7	{ fresh necrotic	0.45 —	1.14 —	0.80 —
50 : 2	8	{ fresh necrotic	0.21 —	0.37 —	0.31 —
50 : 3	33	{ fresh necrotic	0.14 0.073	0.13 0.063	0.33 0.23
Mean value		{ fresh necrotic	0.47 0.23	0.63 0.26	0.65 0.16

Percentage of injected P^{32} present in 1 mgm

	Liver P	Plasma P	Sarcoma P		Nucleic acid P	
			fresh	necrotic	fresh	necrotic
49 : 1	1.53	0.92	1.15	0.67	0.0083	0.0059
49 : 2	0.64	1.00	0.82	0.58	0.012	0.0026
49 : 3	1.71	1.18	1.19	1.17	0.012	0.00043
49 : 4	6.23(?)	1.60	1.48	1.27	0.0077	—
50 : 1	1.69	0.74	1.05	0.25	0.0084	—
50 : 2	2.04	1.15	1.38	—	0.0043	—
50 : 3	1.29	1.50	0.55	0.42	0.0018	0.00095
Mean value	2.16	1.16	1.09	0.73	0.0078	0.0025

The intrinsically decreased metabolism of nucleic acid in necrotic tissue is reduced still further by the action of the X-rays.

TABLE 10. — COMPARISON OF THE MEAN VALUES OF NUCLEIC ACID FORMATION AND OF PHOSPHATE PERMEABILITY IN FRESH AND NECROTIC TISSUE

		Fresh tissue	Necrotic tissue
Activity of 1 mgm of nucleic acid phosphorus as a percentage of the activity of the free sarcoma P	Unirradiated	2.05	1.12
	Irradiated with 2000 r	0.65	0.16
Ratio of the activity of 1 mgm of free P of the necrotic and fresh sarcoma tissues ...	Unirradiated	0.66	
	Irradiated	0.76	

Amount of Nucleic Acid Newly Formed in the Sarcoma Tissue in the Course of 2 hr

We found (Table 8) that two hours after subcutaneous injection of the radioactive phosphate, the activity of the nucleic acid phosphorus amounts to 2 per cent of the activity of the free sarcoma phosphorus. If the activity of the free sarcoma phosphorus were the same during the experimental period as at the end of the experiment, it would be possible to conclude from the above data that nucleic acid molecules constituting 2 per cent of the total nucleic acid present in the sarcoma, i. e. on the average 0.18 mgm/g of tissue for sarcomas weighing less than 40 gm (see Table 12), had been formed during the 2 hr experimental period. The activity of the sarcoma P, however, changes in the course of the experiment. If we assume, for example, that it increases linearly with

time, then the percentage of newly formed nucleic acid molecules is not 2 per cent but 4 per cent. The change in the specific activity of the free phosphorus in the sarcoma cells does not follow a simple proportionality; initially it is practically equal to zero for a few minutes, since the absorption of the injected P and its penetration into the extracellular and intracellular volumes require time. On the other hand, the specific activity rises only slightly with time in the last phase of the experiment; when equilibrium between the activity of the plasma (extracellular) P and the cellular P is almost attained, then the specific activity of the sarcoma P changes only slightly with time. In a number of cases has there been not only equalization of the specific activities of the sarcoma and liver phosphorus after two hours, but the former has indeed exceeded the latter. The cause of this process is that the activity of the plasma P reaches a maximum within the first $\frac{1}{2}$ hr after subcutaneous injection and then decreases gradually. Very active P penetrates into the cells from the highly active plasma; this P is, of course, again replaced by newly arriving less active plasma P, but the equalization of activity between the sarcoma P and plasma P takes place more slowly than the changes in the plasma activity, and thus is explained the fact that sarcoma P more active than plasma P can be encountered after 2 hr. In the above discussions it must be borne in mind that the free phosphorus of the sarcoma cells has not only the ability to enter and escape through the cell wall, but also has various chances of incorporation into the organic phosphorus-containing molecules of the sarcoma cells. After the entry of the very active plasma P there is a correspondingly rapid entry of P into adenosine triphosphate, hexose monophosphate and similar molecules, which then serve as a storage space of the highly active P. If this phosphorus flows back again into the plasma, which has meanwhile become impoverished, then the loss in activity of the free sarcoma P will be compensated by an escape of strongly active P from the storage space and, therefore, this process contributes to maintaining the higher level of activity of the free sarcoma P. In certain conditions the activity of the sarcoma P can indeed undergo a decline, instead of a nonlinear increase with time, in the later phases of the experiment. By multiplying the final value of the specific activity of the sarcoma P by about 1.5, we should obtain the average value for the free sarcoma P during the course of the experiment and of the raw material serving for the formation of the active nucleic acid. We then have to multiply by 1.5 the value obtained for the ratio of the activities of 1 mgm of nucleic acid P and 1 mgm of sarcoma P in order to obtain the value of the percentage increase of nucleic acid in the sarcoma in the course of 2 hr. This value therefore amounts to about 3 per cent of the total quantity of nucleic acid, or on the average 0.26 mgni/gm of sarcoma.

Growth and Renewal

The nucleic acid formed during the course of the experiment, and for that reason radioactively labelled (containing ^{32}P), is either to be found in the newly formed tissue or to be attributed to the renewal of nucleic acid already present. In the case of adenosine triphosphoric acid and some other acid-soluble phosphorus compounds, renewal of the molecules in the sarcoma and in other organs takes place at a very high rate. These compounds exhibit radioactivity after the passage of only a very short time when radioactively labelled phosphate is present. The nucleic acid molecules, on the other hand, are only very slowly renewed in the normal organs. Data on the speed of renewal of nucleic acid in the organs of adult rats will be communicated shortly.

We assume that the nucleic acid content of the sarcoma is proportional to its weight or volume. This assumption is supported by the observations which are discussed below. The percentage growth of the nucleic acid content is then equal to the percentage volume increase of the sarcoma. By means of the radioactive experiments, we determine the percentage of nucleic acid molecules which have been formed in the period of 2 hours before the rat is killed and we ascertain the growth by studying the volume increase of the sarcoma in the same period. The size, however, is too small to be ascertained by measuring the dimensions of the sarcoma. The growth which has taken place in the last 24 hr, on the other hand, can be measured and hence the growth occurring in the last 2 hr can be calculated. It seems to be more correct, however, to base the calculation of the volume increase in the 2 hr period not on a single measurement, which is affected by uncertainties (see Table 11), but to use a series of measurements which have been made over the last 6 days of investigation. The results of these and other measurements are to be seen in Tables 11 and 12.⁽¹⁾ The latter table contains a summary of the results obtained for sarcomas which weigh less and more than 40 gm. The sarcomas studied by the radioactive methods were mostly lighter than 40 gm and our interest is therefore more particularly in the volume increase of this group of sarcomas.

The dry weight of the sarcomas studied varied between 18.5 and 20.7% (average 19.2%) of the weight of the sarcoma.

It is readily appreciated that in sarcomas which have nearly attained the limit of their possible growth, the daily percentage volume increase is considerably less than in sarcomas which are able to grow to an almost unlimited extent. The average content of nucleic acid per gm of sarcoma in both cases, however, is found to be essentially the same; it amounts

¹ Values of 26.5 and 14.3, respectively, are obtained by calculating the percentage volume increase of the sarcoma from measurements taken at the beginning and end of the last day.

TABLE 11. — INCREASE IN VOLUME OF THE SARCOMA

Rat	Weight and specific weight of the sarcoma; total nucleic acid content per gm	Date	Volume of sarcoma ⁽¹⁾ (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
48 : 1a	15 gm; (2.5) 3.6 mgm	16/5	2.77	—	25.0 ²
		18/5	4.88	38.1	
		19/5	5.63	15.3	
		20/5	6.10	8.4	
48 : 2a	8 gm; (2.3) —	18/5	0.80	—	34.4
		19/5	1.13	41.2	
		20/5	1.62	43.2	
		21/5	2.16	33.4	
		22/5	2.49	15.3	
		23/5	3.46	38.8	
48 : 3a	8 gm; (2.4) 4.9 mgm	18/5	1.09	—	27.9 ³
		19/5	1.59	46.0	
		20/5	2.64	66.6	
		21/5	2.64	0	
		22/5	3.35	26.8	
		23/5	3.35	0	
48 : 1b	19 gm; (2.5) 9.5 mgm	26/5	1.42	—	30.5
		27/5	2.01	41.5	
		29/5	3.81	44.8	
		3/05	4.23	11.0	
		1/6	6.03	21.5	
		2/6	7.75	28.5	
48 : 2b	28 gm; (2.0) 7.1 mgm	26/5	3.23	—	20.3
		27/5	5.24	62.3	
		29/5	7.37	20.4	
		30/5	8.77	19.0	
		1/6	14.25	31.1	
		2/6	14.25	0	
48 : 5b	11 gm; (1.8) 12.6 mgm	29/5	0.84	—	28.0
		30/5	1.42	69	
		1/6	1.80	13.4	
		2/6	2.47	37	
		3/6	3.52	42.6	
		4/6	4.53	28.7	
		5/6	6.03	33.1	

¹ The volume of the sarcoma was calculated from the length, breadth and depth, assuming an elliptical form of the sarcoma.² Increase observed for 4 days only.³ Increase observed for 5 days only.

TABLE 11. — (*contd.*)

Rat	Weight and specific weight of the sarcoma; total nucleic acid content per gm	Date	Volume of sarcoma ⁽¹⁾ (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
48 : 6b	15 gm; (1.7) 12.3 mgm	26/5	0.34	—	22.7
		27/5	1.01	197	
		29/5	1.17	7.9	
		30/5	1.34	14.5	
		1/6	1.34	0	
		2/6	1.84	37.3	
		3/6	2.89	57	
		4/6	2.80	0	
		5/6	3.81	36.1	
		6/6	4.53	18.7	
		8/6	6.54	22.2	
		9/6	8.97	34.2	
48 : 12b	26 gm; (1.9) —	9/6	1.8	—	36.3
		13/6	6.12	60	
		15/6	11.28	42	
		16/6	14.08	25.	
48 : 13b	20 gm; (1.9) —	9/6	1.93	—	24.0
		13/6	5.7	49	
		15/6	7.84	18.8	
		16/6	10.56	34.5	
49 : 1	36 gm; (2.0) 9.1 mgm	18/5	1.93	—	23.6
		19/5	2.81	45.7	
		20/5	3.69	31.4	
		21/5	5.36	45.3	
		22/5	6.34	18.8	
		23/5	8.13	27.6	
		26/5	13.49	22.0	
		27/5	13.49	0	
		28/5	18.44	36.9	
49 : 3	20 gm; (2.1) 9.3 mgm	18/5	1.47	—	25.0
		19/5	1.84	25.0	
		20/5	2.60	52.2	
		21/5	3.18	22.5	
		22/5	3.77	18.5	
		23/5	5.24	38.8	
		26/5	7.04	11.4	
		27/5	11.0	56.4	
		28/5	13.28	20.7	

Rat	Weight and specific weight of the sarcoma; total nucleic acid content per gm	Date	Volume of sarcoma ⁽¹⁾ (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
49 : 4	30 gm; (1.6) 13.7 mgm	18/5	1.51	—	21.5
		19/5	2.43	61.0	
		20/5	3.27	34.5	
		21/5	4.69	43.4	
		22/5	6.37	35.9	
		23/5	9.05	42.0	
		26/5	11.56	9.2	
		27/5	16.13	39.9	
		28/5	19.32	19.6	
50 : 1	18 gm; (2.5) 9.9 mgm	4/6	2.6	—	35.6
		9/3	7.25	35.6	
50 : 2	18 gm; (2.1) 4.6 mgm	4/6	3.35	—	30.2
		9/6	8.40	30.2	
48 : 3b	51 gm; (1.7) 13.0 mgm	26/5	2.93	—	21.3
		27/5	4.32	47.8	
		29/5	8.46	47.7	
		30/5	12.57	21.7	
		1/6	14.54	7.8	
		2/6	15.50	6.6	
		3/6	18.81	18.2	
		4/6	20.53	9.1	
		5/6	29.30	42.7	
48 : 4b	42 gm; (1.9) 14.5 mgm 14 mgm	26/5	2.43	—	19.4
		27/5	4.48	84.4	
		29/5	5.49	11.3	
		30/5	8.46	53.3	
		1/6	11.10	15.0	
		2/6	13.20	18.9	
		3/6	15.50	17.4	
		4/6	21.37	37.9	
		5/6	22.63	5.9	
48 : 7b	75 gm; (1.9) 14.5 mgm	26/5	2.51	—	16.0
		27/5	4.06	6.16	
		29/5	8.42	53.5	
		30/5	9.23	9.6	
		1/6	14.71	30	
		2/6	16.97	15.4	
		9/6	17.18	1.2	

TABLE 11. — (*contd.*)

Rat	Weight and specific weight of the sarcoma, total nucleic and content per gm	Date	Volume of sarcoma(¹) (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
48 : 7b (<i>contd.</i>)	75 gm; (1.9) 14.5 mgm	4/6	22.46	—	12.8
		5/6	26.4	17.5	
		6/6	29.84	13.1	
		8/6	36.84	11.6	
		9/6	40.56	10.0	
48 : 8b	81 gm; (1.6) 10.8 mgm	26/5	4.69	—	7.1
		27/5	6.37	35.9	
		29/5	7.75	10.2	
		30/5	10.89	40.5	
		1/6	15.71	31.5	
		2/6	20.66	15.2	
		3/6	21.34	3.4	
		4/6	27.15	27.0	
		5/6	35.95	32.0	
		6/6	34.22	0	
		8/6	38.17	5.6	
		9/6	42.54	11.5	
		10/6	49.15	15.4	
		11/6	51.29	4.3	
		12/6	51.29	0	
48 : 9b	79 gm; (1.5) —	26/5	3.52	—	11.9
		27/5	4.99	42	
		29/5	7.0	21	
		30/5	10.39	49	
		1/6	13.67	15.5	
		2/6	19.19	41.1	
		3/6	23.51	22.5	
		4/6	29.41	25.3	
		5/6	29.86	1.5	
		6/6	31.68	6.0	
		8/6	41.36	15.1	
		9/6	37.75	0	
		10/6	47.85	29.2	
		11/6	45.77	0	
		12/6	51.33	12.1	
48 : 10b	83 gm; (1.7) —	26/5	2.35	—	35.4
		27/5	3.69	57.2	
		29/5	5.53	25	

Rat	Weight and specific weight of the sarcoma; total nucleic acid content per gm	Date	Volume of sarcoma ¹ (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
48 : 10b (contd.)	83 gm; (1.7) —	30/5	6.83	23.5	8.7
		1/6	8.84	14.7	
		2/6	9.51	7.6	
		3/6	12.07	26.8	
		4/6	16.89	40.0	
		5/6	19.90	17.7	
		6/6	20.82	4.6	
		8/6	26.15	12.8	
		9/6	26.15	0	
		10/6	29.87	2.8	
		11/6	34.91	16.8	
		12/6	39.13	12.1	
		13/6	39.13	0	
		15/6	41.94	3.6	
		16/6	48.27	14.8	
48 : 11b	78 gm; (1.8) —	26/5	1.13	—	8.4
		27/5	1.76	56.0	
		29/5	2.81	30.0	
		30/5	7.04	93.0	
		1/6	9.22	15.5	
		2/6	11.31	22.6	
		3/6	15.25	34.7	
		4/6	16.89	11.4	
		5/6	18.77	11.1	
		6/6	23.59	25.6	
		8/6	31.68	17.1	
		9/6	34.32	8.2	
		10/6	35.2	2.6	
		11/6	26.29	3.1	
		12/6	41.4	14.4	
		13/6	43.45	5.0	
49 : 2	44gm; (1.9) 10.1 mgm	16/5	3.02	—	12.7
		18/5	4.99	33	
		19/5	6.29	26.1	
		20/5	7.25	15.3	
		21/5	9.30	27.7	
		22/5	11.44	24.1	
		23/5	11.44	0	

TABLE 11. — (*contd.*)

Rat	Weight and specific weight of the sarcoma; total nucleic acid content per gm	Date	Volume of sarcoma ⁽¹⁾ (cm ³)	Daily per cent volume increase	Average of daily percentage volume increase in the course of the last 6 days
49 : 2 (<i>contd.</i>)	64 gm; (1.9) 10.1 mgm	26/5	17.30	17	13.4
		24/5	20.91	20.8	
		28/5	23.09	10.2	
50 : 3	64 gm; (1.9) 10.2 mgm	26/5	3.44	—	12.2
		27/5	6.45	79	
		29/5	7.42	15.0	
		30/5	9.13	23.9	
		1/6	12.19	16.8	
		2/6	15.08	24.0	
		3/6	17.56	14.5	
		4/6	20.11	16.4	
		5/6	21.24	5.6	
		6/6	27.36	28.8	
		8/6	33.44	11.1	
		9/6	33.44	0	

to 8.8 and 12.0 mgm/gm of sarcoma, respectively. This finding, which relates to two groups of sarcoma with very different sizes, supports the correctness of our assumption that the percentage increase in nucleic acid runs approximately in parallel with the percentage volume (weight) increase.

TABLE 12

Average volume increase of 14 and 10 sarcomas within day	Average nucleic acid content per gm of sarcoma
Sarcomas weighing less than 40 gm	
27.4%	8.8 mgm
Sarcomas weighing more than 40 gm	
12.8%	12.0 mgm

The average specific weight of the heavy sarcomas varies between 1.5 and 1.9 gm with a mean of 1.8; the corresponding limits for the sarcomas weighing less than 40 gm are 1.6 and 2.5; the mean value for these amounts to 2.1.

For the sarcomas in which we are interested the increase in volume and the corresponding increase in nucleic acid amount to 27 per cent daily, or about 2 per cent in the course of 2 hr.⁽¹⁾ The newly formed quota

¹The above result must be interpreted with care since the volume increase of the sarcoma is possibly rhythmical during the course of the day.

of nucleic acid was obtained from the radioactive measurements within about 3 per cent. A very substantial fraction of the radioactively labelled nucleic acid must therefore be ascribed to the growth process.

Effect of X-rays on the Formation of Acid-Soluble Phosphorus Compounds in the Sarcoma

In contrast to the synthesis of nucleic acid molecules, the formation of acid-soluble phosphorus compounds in the sarcoma is not noticeably affected when exposed to a dosage of a few thousand roentgens, as is shown in Table 13.

TABLE 13. — FORMATION OF RADIOACTIVELY LABELLED ACID-SOLUBLE PHOSPHORUS COMPOUNDS IN THE IRRADIATED SARCOMA

Rat	Dosage (r)	Fraction denoted by its hydrolysis time of (min)	Percentage of injected ^{32}P per mgm P
I	1395	0	100
		7	95
		180	85
II	1670	0	100
		7	88
		180	84
III	2040	0	100
		7	100
		180	84
IV	2000	0	100
		7	100
		100	98
		180	93
V	2000	0	100
		7	85
		100	85
		180	100
VI	2100	0	100
		7	92
		100	98
		180	98

It is evident from Table 13 that the 7-min fraction has nearly the same specific activity as the 0-min fraction, and that the bulk of the remaining fractions is also radioactively labelled, i. e. it has been newly

formed in the course of the experiment. X-ray dosages of 2000 r units do not, therefore, prevent the almost complete renewal of the molecules of hydrolysable acid-soluble phosphorus compounds in the sarcoma. The metabolism of the acid-soluble phosphorus compounds is closely related to the oxidation and reduction processes taking place in the cells, and it is well known that these latter processes are quite insensitive towards the action of X-rays.⁽¹⁾

Enzyme Activity in Irradiated Jensen-Sarcomas

An effect of X-rays on the enzymes taking part in the processes of degradation and synthesis of the acid-soluble phosphorus compounds was not to be expected, having regard to the insensitivity of these processes to X-rays, as mentioned in the last section.

Experiments in which the effectiveness of the catalase isolated from the sarcoma 1 hr after irradiation with 3000 r was studied, showed that this effectiveness had not suffered as a result of irradiation of the sarcoma. While the catalase activity of the muscle of normal rats has been found to be 0.0252, the muscle catalase of the irradiated rats showed a value of 0.0385.⁽²⁾

The effect of nuclease, obtained from sarcoma irradiated with 3000 r, on desoxyribonucleic acid used as substrate did not in any way fall short of the effectiveness of the nuclease obtained from unirradiated sarcoma. Whereas the former decomposed 66 per cent of the desoxyribonucleic acid in the course of 4 hr, the corresponding value for the unirradiated sarcoma amounted to 46 per cent.

From the chemical point of view the cell division is a consequence of the very intensive synthetic process which takes place in the nuclei of the cells. Interference with these synthetic processes can stop the cell division. Interference by means of the action of irradiation can take place either by more rapid destruction of the molecules indispensable in the synthesis than the rate at which they are formed, or by the formation of noxious products, as a result of the effect of radiation, which enter into the chemical processes occurring in the cell nuclei or in certain parts of these nuclei and thus inhibit the normal course of these processes and their sequel, cell division. Among the effects of X-rays there is, for example, splitting of high polymer molecules, as is known from the

¹ Whereas four-fifths of the sarcomas have been prevented from growing after transplantation, by irradiation with 1800 r, the oxygen consumption of these sarcomas was not different from that of the control sample (W. KEIL, *Arch. exp. Pathol.*, **167**, 338 (1932).

² These experiments are described in more detail elsewhere.

work of SVEDBERG and BROHULT⁽¹⁾; or degradation of plasma proteins into particles of lower molecular weight.

In addition to the cleavage fragments from high polymers, a series of other constituents are found in irradiated tissue such as, for example, nascent oxygen which permits the formation of hydrogen peroxide and other oxidation products.

The X-rays have no selective affinity for one or other type of molecule in the tissue; any atom in the irradiated tissue has approximately the same probability of being ionized as any other atom; it can be assessed at 4×10^{-11} for irradiation with a dose of 1 r.⁽²⁾ It is possible that the X-rays and the ions liberated by the radiation act directly upon a constituent required for the synthesis of nucleic acid, e. g. by cleaving such a molecule; it is not less probable, however, that the entry of noxious products into the synthetic phase leading to cell division is the determining step.

Summary

Rats with Jensen-sarcoma are injected subcutaneously with a radioactively labelled phosphate solution; after the passage of 2 hr desoxyribonucleic acid of the sarcoma is isolated.

If the nucleic acid is found to be radioactive it follows that nucleic acid molecules have been synthesized in the sarcoma during the course of the experiment.

A comparison of the activity of 1 mgm of nucleic acid P with the activity from 1 mgm of free sarcoma P makes it possible to determine the amount of newly formed nucleic acid.

The nucleic acid molecules formed in the course of 2 hr amount to 2—3 per cent of the total nucleic acid content, which on the average constitutes 9 mgm/gm of sarcoma.

The irradiation of the sarcoma with 1000 international roentgen units and even with a dose of 450 r or less, causes a decrease of the formation of nucleic acid, in the great majority of cases, to an average of half to one-third of the value found in an unirradiated sarcoma. Individual sarcomas (five of forty five), which presumably are particularly resistant to radiation, exhibit normal formation of nucleic acid even after irradiation.

The method described permits detection of the effect of radiation on the growth of the sarcoma by a chemical method directly after irradiation.

The nucleic acid formation in necrotic sarcoma tissue amounts to about half to one-quarter of that found in fresh tissue. The free phosphate of the sarcoma cells is replaced more slowly by plasma phosphate in necrotic tissue than in fresh tissue, but in the necrotic tissue the bulk of the free phosphate originally present in the sarcoma cells is also replaced in a period of 2 hr.

¹ TH. SVEDBERG and S. BROHULT, *Nature* **143**, 938 (1938).

² P. JORDAN, *Radiologica* **2**, 25 (1938).



One milligram of the acid-soluble organic P of the sarcoma, which is decomposed in the course of 180 min hydrolysis, shows nearly the same content of radioactively labelled phosphorus, 2 hr. after injection, as 1 mgm of free P of the sarcoma. Almost all molecules of the above mentioned acid-soluble phosphorus compounds, of the sarcoma are thus renewed in a period of 2 hr. Irradiation with a dose of 2000 r does not produce any detectable effect on the rate of renewal of the acid-soluble P compounds of the sarcoma.

72. THE EFFECT OF X-RAYS ON NUCLEIC ACID FORMATION IN THE ORGANS OF THE RAT

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ON an earlier occasion⁽¹⁾ we found that the synthesis of desoxyribonucleic acid molecules in the Jensen-sarcoma of the rat is inhibited by the action of X-rays. We have extended our studies to the effect of radiation on the metabolism of nucleic acid in the normal organs of the rat and we describe the outcome of these experiments in the following pages.

EXPERIMENTAL

Albino rats with ages between 3 days and 12 months were available for this work. In order to obtain sufficient amounts of nucleic acid it was necessary simultaneously to work with organs of several animals, because the requisite detailed purification of nucleic acid from other phosphorus-containing compounds is accompanied by considerable losses of material. As we described previously⁽¹⁾, the nucleic acid was purified with hydrochloric acid and methanol, three times with the addition of phosphate and then twice more without. One-fifth of the nucleic acid obtained was used for measuring the radioactivity and another fifth for colorimetric determination of phosphorus.

The radioactive phosphate was administered to the rats by subcutaneous injection at the beginning of the experiment, using 0.1 cm³ of a physiological saline solution which contained some active sodium phosphate. The dose administered to one rat had an activity of from 3 to 6 μ c. The animals were killed two hours after injection.

The irradiated animals were given the injection immediately after irradiation. The irradiation dose varied between 1300 and 3000 r. The X-ray tube was operated at a voltage of 165 kV. A 0.5 mm copper foil and 1 mm aluminium foil were used as radiation filters. The field size was 10 \times 10 cm².

1. H. EULER and G. HEVESY, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **17**, No. 8 (1942); *Ark. Kem.* **17A**, No. 30 (1944).

An average sample of the tissue from the organ was used for isolating the free P in the organ. In some instances the total P content of the organs also was isolated. In these fractions also, one-fifth was used for determining the radioactivity and one-fifth for colorimetric determination of the P content. The radioactivity was measured by means of a Geiger—Müller counter.

EXPERIMENTAL RESULTS

The ratio of the ^{32}P content of 1 mgm of nucleic acid P at the end of the experiment to the ^{32}P content present on the average in 1 mgm of free P in the organ during the experiment was used as a measure of the rate of formation of nucleic acid. The ^{32}P content of the free P in the organ was determined at the end of the experiment. The values quoted in the tables are, therefore, not an exact measure of the nucleic acid formation. The rate of formation of nucleic acid in the organs of the rat was determined in collaboration with J. OTTESEN⁽¹⁾, taking into account the mean activity of free P prevailing during the experiment. In the present study it was desired to investigate the effect of X-rays on the rate of formation of nucleic acid, and for this purpose it is sufficient to compare the activity of the nucleic acid P at the end of the experiment with the activity of the free P in the organs at the same time. As we shall discuss later (p. 725), the difference between the final and average activities of the free P in the organs involved is not very considerable and in the case of the liver is almost negligible.

Table 1 exhibits the rate of formation of nucleic acid in the organs of the unirradiated rat. Table 2 show the metabolism of nucleic acid determined in the organs of the irradiated rat.

We have not included any of the results obtained on 5-week old rats in Table 1 since no rats of this age have been irradiated; the figures in Table 1 should be compared with those in Table 2, which contains data on irradiated rats.

The results which have been obtained with 5-week old rats are presented in Table 1a.

Finally, a study was made of the rate of formation of nucleic acid in the organs of adult rats which had been starved for 5 days. Table 1b shows that the rate of formation of nucleic acid was not much affected by the fasting.

It has already been demonstrated by one of us in collaboration with J. OTTESEN⁽¹⁾ that the rate of formation of nucleic acid, in contrast to that

¹ G. HEVESY and J. OTTESEN, *Acta Physiol. Scand.* **5**, 237 (1943).

TABLE 1. — FORMATION OF NUCLEIC ACID IN THE ORGANS OF UNIRRADIATED ADULT RATS IN A PERIOD OF 2 hr

Expt. no.	No. of rats	Age and sex	Organ	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of	
				Free P in the organ	Plasma P
80	8	1 year F	liver	0.052	0.188
			spleen	1.13	—
			kidneys	0.119	0.218
82	8	3 months F	liver	0.20	0.12
83	8	1 year F	liver	0.034	0.069
			kidneys	0.070	0.077
84	8	10 months F	liver	0.031	0.049
			kidneys	0.14	0.13
85	8	15 months F	liver	0.054	0.064
			spleen	1.41	1.00
			kidneys	0.19	0.18
88	8	6 months F	liver	0.32	0.12
			spleen	3.57	2.2
93	10	7.5 months F	liver	0.26	0.30
			kidneys	0.15	0.14
97	15	11 months F	liver	0.070	0.103
			spleen	3.19	2.51
			kidneys	0.18	0.26
97	15	11 months F	intestinal mucosa ...	5.05	2.40
99	20	5 months F	liver	0.21	0.18
			spleen	—	1.29
			kidneys	0.25	0.21
			intestinal mucosa ...	6.8	2.6
103	5	5 months F	spleen	2.9	1.97
			intestinal mucosa ...	2.4	0.87
151 I	1	6 months F	liver	0.13	0.31
151 II	8	3 months F	liver	0.11	0.13
Average values			liver	0.136	0.203
			spleen	2.50	1.79
			kidneys	0.158	0.15
			intestinal mucosa ...	1.8	2.1

of most of the acid-soluble phosphorus compounds and phosphatides, in the liver is very small and also that the spleen and especially the intestinal mucosa are characterized by a high formation of nucleic acid; ANDREASEN and OTTESEN⁽¹⁾ have shown recently that the metabolism of nucleic acid in the thymus exceeds that in all other organs and is twice as large in this organ as in the small intestinal mucosa.

¹ E. ANDREASEN and J. OTTESEN, *Acta Path. Microbiol. Scand.* Suppl. 54. (1944).

TABLE 1a. — FORMATION OF NUCLEIC ACID IN THE ORGANS OF 5 WEEK OLD RATS

No. of animals	Age and sex	Organ	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of	
			Free P in the organ	Plasma P
22	5 weeks F	liver	0.059	0.02
		spleen	4.8	1.1
		kidneys	0.159	0.18
20	5 weeks F	liver	0.24	0.32
		spleen	2.9	2.2

TABLE 1b. — FORMATION OF NUCLEIC ACID IN THE ORGANS OF STARVED ADULT RATS

No. of animals	Age and sex	Organ	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of	
			Free P in the organ	Plasma P
6	6 months M	liver	0.23	0.20
		spleen	1.08	0.62
		kidneys	0.29	0.20
		intestinal mucosa	8.5	3.9
7	2—4 months M	liver	0.22	—
		spleen	0.93	—

As we have already stated, the ratio of the specific activity of the nucleic acid P at the end of the experiment to the specific activity of the free P at this same time is not a correct measure of the nucleic acid molecules newly formed during the experiment. An accurate calculation of this quantity requires a knowledge of the average specific activity of the free P in the course of the experiment. With regard to the liver, of course, these last mentioned quantities are very little different from the values which have been obtained for unirradiated rats, as is shown by the following data which we obtained on a previous occasion⁽¹⁾.

The above figures prove that the average specific activity of the free P in the 2 hr experiment is only about 5% less than the final activity. The number 0.13 in the last horizontal line of Table 1 should, therefore, be multiplied by 1.05 in order to arrive at the percentage rate of nucleic acid formation in the liver in a period of 2 hr. Similar conditions are involved in the kidneys, where the added phosphate ions likewise pene-

¹ H. EULER and G. HEVESY *Ark. Kem.* **17A**, No. 30 (1944).

trate rapidly into the cells and then gradually leave again. The rate of penetration of the labelled phosphate into the spleen cells is slower and should not differ very considerably from the rate of penetration into the cells of the Jensen-sarcoma, where the final value of the specific activity of the free P was found to be about 25 per cent greater than

Time after injection of ^{32}P (hr)	Percentage of the injected ^{32}P present in 1 mgm of free liver P
$\frac{1}{2}$	2.3
1	2.8
2	2.1

the mean value. If the final value 2.5, in the last horizontal row of Table 1, is multiplied by about 1.25 we approach the percentage renewal of nucleic acid in the spleen taking place in a period of 2 hr.

We also determined the variation in the specific activity of the free phosphorus in the liver and spleen of $3\frac{1}{2}$ day rats with time. The following percentages of the ^{32}P injected into each rat (0.05 cm³ of radioactively labelled physiological sodium phosphate solution), were found in 1 mgm of free P as shown :

Time (hr)	% of injected ^{32}P present in 1 mgm of free liver P	% of injected ^{32}P present in 1 mgm of free spleen P
$\frac{1}{2}$	2.12	2.13
1	2.39	2.19
2	2.11	2.17

In these experiments also, the final value of the specific activity of 1 mgm of free liver P differs only a little from the mean value of the activity during the experiment. A comparison of the values obtained for the spleens of $3\frac{1}{2}$ day rats with those for adult rats (cf. Table 3) yields the result that the phosphate ions penetrate into the spleen cells of $3\frac{1}{2}$ day old rats at about the same rate as into the liver cells, whereas the liver cell walls of the adult animals are appreciably more permeable to phosphate than are the spleen cell walls.

Effect of Irradiation on the Rate of Formation of Nucleic Acid

Table 2 contains data for the rate of formation of nucleic acid in the organs of irradiated rats. The animals were exposed to total body irradiation, except that the head was covered with 5 mm thick lead sheet. The time of irradiation amounted to 20–40 min. Irradiation was performed with

165 kV X-rays. A 0.5 mm copper foil and a 1 mm aluminium foil were used as radiation filters. The rats were irradiated at a distance of 28 cm. The ^{32}P was injected immediately after the end of the irradiation.

TABLE 2. — FORMATION OF NUCLEIC ACID MOLECULES IN THE ORGANS OF IRRADIATED ADULT RATS

Expt. no.	No. of rats	Age and sex	Dose (r)	O r g a n	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of	
					Free organ P	Plasma P
100	10	5 months	1480	liver	0.034	0.061
				spleen	0.55	0.49
				intestinal mucosa ...	1.7	1.2
103	5	5 months	1480	liver	—	0.18
				spleen	1.2	0.89
				intestinal mucosa ...	2.6	0.78
104	6	9 months	1480	liver	0.036	0.044
				spleen	—	—
				intestinal mucosa ...	1.5	0.8
129	3	9 months	2000	liver	0.038	0.056
				spleen	0.37	0.20
138	3	5½ months	3000	liver	0.096	0.133
				spleen	1.90	1.45
137	3	10 months	3000	liver	0.049	0.069
				spleen	0.738	0.51
		Mean values		liver	0.0506	0.090
				spleen	0.952	0.708
				intestinal mucosa ...	1.9	0.9

Expt. no.	No. of rats	Age and sex	Dose (r)	O r g a n	Activity of 1 mgm of nucleic acid P as a percentage of the activity in 1 mgm of	
					Free organ P	Plasma P
115 ¹	2	3 months	1300	liver	0.337	0.353
				spleen	2.08	1.38

¹ Two rats having sarcoma were used in this experiment, the whole body being protected, except the sarcoma with an area amounting to $5 \times 3.5 \text{ cm}^2$, with a 5 mm thick lead sheet.

A comparison of the activity of 1 mgm of nucleic acid P in unirradiated rats, as a percentage of the activity of 1 mgm of free phosphorus in the organ or plasma, with the corresponding figures for unirradiated animals (cf. Table 3) shows that the formation of nucleic acid in the organs which have been studied is inhibited by irradiation. Consequently, the effect of X-rays which has been observed on the sarcoma and which inhibits the formation of nucleic acid, extends to the non-growing normal organs of adult animals.

TABLE 3. — COMPARISON OF THE ACTIVITY OF 1 mgm OF NUCLEIC ACID P IN UNIRRADIATED RATS, EXPRESSED AS A PERCENTAGE OF THE ACTIVITY OF 1 mgm OF FREE PHOSPHORUS IN THE ORGAN OR PLASMA, WITH THE CORRESPONDING VALUE IN IRRADIATED RATS

Organ	Ratio: unirradiated—irradiated	
	Compared with free P in the organ	Compared with free P in the plasma
Liver	3.3	2.3
Spleen	2.4	2.5
Intestinal mucosa	2.3	2.3

Formation of Nucleic Acid in the Liver of 3½—4½-day old Unirradiated and Irradiated Rats

We have extended our investigations to the determination of the nucleic acid formation rate in the liver and spleen of strongly growing 3—4 day old rats.

Twenty-seven rats aged 3½ days were each injected with 0.05 cm³ of a solution containing ³²P (activity 2 μ c). After 2 hr the rats were killed and the nucleic acid P was isolated, as also the free P, from the liver and spleen. The formation of nucleic acid, determined by means of the activities of these fractions, can be seen in Table 4.

TABLE 4. — FORMATION OF NUCLEIC ACID IN THE ORGANS OF TWENTY-SEVEN 3½-day Old Rats in a Period of 2 hr

Organ	Crude nucleic acid (mgm)	Separated pure nucleic acid (mgm)	P content of the nucleic acid %	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of free P in the organ
Liver	76.0	9.3	7.21	1.96
Spleen	20.1	2.1	7.95	9.76

In order to determine the effect of X-rays on the formation of nucleic acid in the liver of strongly growing rats, we irradiated seven 3—4-day old rats with 2000 r immediately before injecting with radioactive phosphate. The rate of formation of nucleic acid during a period of 2 hr was found to be 0.81 per cent and therefore less than half the value (1.96) determined in the unirradiated animals.

This magnitude of nucleic acid formation in the liver of unirradiated or irradiated *strongly growing* (4-day old) rats is an order of magnitude greater than the formation found in the liver of adult rats. The per-

centage inhibition of the nucleic acid formation due to the action of X-rays is, on the contrary, about the same in the liver of strongly growing and adult rats and, furthermore, it is not greatly different from the percentage inhibition observed in the case of the Jensen-sarcoma.

Summary

The metabolism of desoxyribonucleic acid has been determined in the organs of 250 rats, aged between 3 days and 1 year, by using radioactively labelled phosphorus as an indicator, and a study has been made of the effect of X-rays on the rate of nucleic acid formation.

Immediately after irradiation of adult rats with from 1500 to 3000 r, the formation of new molecules of desoxyribonucleic acid in the liver, spleen and intestinal mucosa is found to be reduced in the course of 2 hr to one-third to one-half, i.e. to an extent similar to that found in the Jensen-sarcoma. The percentage of newly formed desoxyribonucleic acid, in a period of 2 hr, in the liver and spleen of $3\frac{1}{2}$ -day old rats is forty and ten times, respectively, the amount found in the corresponding organs of adult animals.

The percentage reduction in the formation of nucleic acid in the organs of 3—4-day old rats due to the action of X-rays is not greatly different from that observed in the organs of adult rats.

COMMENT ON PAPERS 71 AND 72

THAT DNA is built up prior to cell division was inferred at that date (1939) when the study, the result of which is communicated in paper 71, was initiated. A suppression of DNA formation should thus lead to a mitotic arrest, and vice versa and a suppression of DNA formation under the effect of exposure to irradiation should reflect itself in a depressed ^{32}P incorporation into the DNA molecules formed in the tissue of rats studied (papers 71 and 72 and EULER and HEVESY, 1944). To determine a change of 1 per cent in the DNA content of a tissue with cytochemical methods, difficulties are encountered even today (in the meantime great progress was made in this field). The tracer method is very suitable for demonstrating small differences in the new-formation of DNA molecules. If during the experiment in the non-exposed Jensen sarcoma 1 per cent of the DNA molecules get labelled (thus newly formed) a formation of 0.96 per cent only in the irradiated sarcoma (thus a difference of 0.04 per cent in the total DNA content) will be easily detectable. That DNA is built up prior to cell division was first proved by HOWARD and PELC (1951). In *Vicia faba* which they investigated, the synthesis was already found to be terminated 6 hr prior to the beginning of visible prophase. They made use of the powerful autoradiographic method in their investigations.

After irradiation with a Roentgen ray dose of 300 r or more, incorporation of ^{32}P into the DNA of the Jensen-sarcoma of most of the few hundred investigated rats (paper 71 and EULER and HEVESY, 1944) was found to be strongly diminished, as well as incorporation into the normal organs of 250 partly growing and partly adult rats (72). The cells of the organs investigated were in different stages of the mitotic cycle, and, correspondingly, the results obtained indicate a resultant of the effect of exposure to radiation on cells which were in different stages of the division process.

HOLMES (1947, 1948) investigated the effect of irradiation with Röntgen rays both on the incorporation of P^{32} into DNA and RNA. These investigations brought out as well, as did very numerous later studies, the blocking effect on ^{32}P incorporation into DNA, while incorporation of ^{32}P into RNA was found by HOLMES to be only slightly affected. The result arrived at in papers 71 and 72 and by EULER and HEVESY (1944) that exposure to Roentgen rays diminished ^{32}P incorporation to about half the value observed in controls, was substantiated by a great number of investigations (as for example, by those of PELC and HOWARD, 1955) published in the course of the last fifteen years, not, however, a possible interpretation of this effect. According to the latter, incorporation of ^{32}P into DNA may be partly due to additional formation of DNA molecules and partly to renewal of DNA molecules already present. It was suggested that irradiation with Roentgen-rays possibly interferes with the formation of additional DNA molecules but not with their turnover. We know today that in the growing tissue the formation of labelled DNA molecules takes place at least in most cases in connection with mitotic processes only. This view was already put forward in the first investigation in this field (paper 67) where it is stated: "The rate of renewal of the nucleic acid molecules in the liver may be identical with the rate of new-formation of liver cells"

Irradiation with ionizing radiation can interfere with DNA formation in different ways. Cell destruction produced by irradiation will stop DNA synthesis.

As first shown by HOWARD and PELC (1953) irradiation can stop cell division even in that part of the interphase in which the DNA synthesis, preceding mitosis, is already terminated. Cell division being blocked, DNA synthesis is bound to stop after the lapse of hours the length of which depends on the system investigated. We meet here a second indirect way of interference with DNA formation. The third way is a direct interference with DNA synthesis. This is due, as made very probable by ORDEN and STOCK and others, to a disturbance of the template, to a macromolecular lesion as denoted by MITCHELL, and also to a change produced in the phosphorylating enzymes. According to the results obtained by LAJTHA *et al.* (1958) investigating ^{14}C incorporation into bone marrow cells, doses below 300 r produce in cells which are in the presynthetic period at the time of radiation a 40–50 per cent depression of the number of cells entering the subsequent synthetic period in a given time, without affecting the rate of subsequent DNA synthesis. As to the 50 per cent depression of DNA formation due to exposure to radiation LAJTHA *et al.* put forward two alternative suggestions. (a) There are two cell populations in the presynthetic phase, one sensitive and one resistant to small doses of radiations. The sensitive cells can be prevented from entering the synthetic period; the resistant ones will enter into and proceed in it undisturbed. (b) All presynthetic cells are sensitive, and the maximum damage results in slowing down the rate of entry into the synthetic period about half. This occurs not in a form of accumulation of presynthetic stage cells just before the beginning of the synthetic period, but more likely by slowing down the “progress through the presynthetic period”. Thus an unambiguous explanation of the 50 per cent depression of DNA synthesis due to exposure to irradiation is still outstanding.

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73. TURNOVER OF NUCLEIC ACID IN RETROGRADE SARCOMATA

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DESOXYRIBOSENUCLEIC acid is wholly or mainly confined to the cell nuclei. That desoxyribosenucleic acid vanishes in certain phases of the mitotic cycle and accumulates in others, was conspicuously shown by CASPERSSON¹ in making use of the technique of ultraviolet absorption. We have, therefore, to expect an appreciable formation of "new" desoxyribosenucleic acid molecules in mitotic cells. The ultraviolet absorption method has the great advantage that it makes it possible to carry out micro-determinations *in situ*. When applying the radioactive method, we have to isolate the desoxyribosenucleic acid P from all other phosphorus fractions present. This requires the employment of substantial quantities of tissues. The radioactive method has, however, 2 advantages:

(a) It indicates all new formation of the desoxyribosenucleic acid, including any such formation which takes place without a perceptible change in the total amount of desoxyribonucleic acid present.

(b) The formation of small amounts of new desoxyribosenucleic acid as correspond to only 0.1 percent, or even less of the total amount of desoxyribonucleic acid present in the tissue sample under investigation, can be determined. The unique sensitiveness of the radioactive method is due to the fact, that the "old" molecules not being radioactive, are not registered at all; and, correspondingly, if the number of molecules increases during the experiment from 1000 to 1001, the radioactivity of the sample increases from 0 to 1. The result arrived at is thus in contrast to all other methods, not based on a small difference between two large figures.

Former investigations into the percentage of new formation of desoxyribosenucleic acid in the growing Jensen sarcomata led to the result that in the course of 2 hours about 2 out of 100 desoxyribosenucleic acid molecules are newly formed.

⁽¹⁾ Cf. T. CASPERSSON and L. Santesson, *Acta Rad. Suppl.* **17** (1942).

Among the numerous Jensen sarcomata obtained by grafting, there were found occasionally those, which showed a spontaneous regression. It seemed to be of interest to investigate if the enzyme cycle leading to the formation of new desoxyribosenucleic acid molecules is undisturbed in such regressive sarcomata, i. e. if the percentage renewal of the desoxyribosenucleic acid molecules present in the regressive sarcoma deviates from the figure obtained in the growing sarcoma, or not.

EXPERIMENTAL RESULTS

In Table 1 the date of inoculation, the weight of the rat and the volume of the sarcoma at different dates is given, thus demonstrating the rate of regression of the sarcomata. Furthermore, the ratio of the activity of 1 mgm desoxyribosenucleic acid P to that of 1 mgm inorganic P from the sarcoma, the plasma and the liver respectively, is stated.

TABLE 1

Experiment 176: I to III

Male rat born $^{15}/_9$ 1944, inoculated with Jensen sarcoma $^{22}/_{12}$

	Date	Weight in gm	Volume of sarcoma length of axes in cm
I.	$^{22}/_{12}$	98	
	$^9/_1$	140	$1.3 \times 2.2 \times 1.5$
	$^{12}/_1$	138	$1.4 \times 2.5 \times 1.5$
	$^{16}/_1$		$1.4 \times 2.6 \times 1.5$
	$^{17}/_1$		$1.3 \times 2.3 \times 1.5$
II.	$^9/_1$	164	$1.1 \times 2.7 \times 1.3$
	$^{12}/_1$	143	$1.2 \times 2.7 \times 1.5$
	$^{16}/_1$		$1.2 \times 2.7 \times 1.3$
III.	$^9/_1$	156	$1.3 \times 2.6 \times 1.4$
	$^{12}/_1$	147	$1.2 \times 2.7 \times 1.4$
	$^{16}/_1$	163	$1.1 \times 2.5 \times 1.3$
	$^{17}/_1$		$1.1 \times 2.35 \times 1.3$

Experiment 177: I to IX.

Male rat born October 1944 inoculated $^{22}/_{12}$

	Date	Weight in gm	Volume of sarcoma axes in cm
I.	$^{22}/_{12}$	105	
	$^9/_1$	157	
	$^{12}/_1$	155	$1.3 \times 2.8 \times 1.5$
	$^{16}/_1$	153	$1.3 \times 3.1 \times 1.7$

	Date	Weight in gm	Volume of sarcoma axes in cm
II.	30/1	180	1.7 × 4.4 × 2.5
	5/2	200	1.8 × 3.5 × 2.7
	6/2		1.6 × 3.5 × 2.6
	17/1	125	1.0 × 2.2 × 1.1
	19/1		0.8 × 2.0 × 1.3
	30/1		1.2 × 2.4 × 1.5
III.	5/2		1.0 × 2.0 × 1.3
	30/1	124	1.3 × 2.7 × 1.7
	5/2	147	1.0 × 2.8 × 1.7
IV.	19/1		1.0 × 2.3 × 1.3
	23/1	146	1.1 × 2.3 × 1.4
	30/1	156	1.0 × 2.2 × 1.3
	5/2	156	0.9 × 2.0 × 1.3
V.	19/1		1.2 × 2.4 × 1.4
	23/1	145	1.4 × 3.1 × 1.7
	30/1	148	1.4 × 2.7 × 1.55
	5/2	170	1.0 × 1.9 × 1.2
VI.	19/1		1.0 × 2.0 × 1.2
	23/1	122	1.1 × 2.2 × 1.2
	30/1	124	1.15 × 2.4 × 1.3
	5/2	136	0.9 × 2.3 × 1.2
VII.	19/1		1.1 × 2.1 × 1.2
	23/1	145	1.2 × 2.2 × 1.2
	30/1	149	1.1 × 2.3 × 1.3
	5/2	160	1.0 × 2.2 × 1.2
VIII.	24/1	161	1.3 × 2.7 × 1.7
	30/-	165	1.5 × 2.7 × 1.9
	5/2		1.0 × 1.9 × 1.4
IX.	30/1	124	1.2 × 2.7 × 1.4
	5/2	133	0.9 × 2.4 × 1.6
	6/2		0.9 × 2.2 × 1.3

Activity of 1 mgm desoxyribosenucleic acid P, expressed in percentage of the activity of 1 mgm inorganic P from the

	sarcoma	plasma	liver
fresh tissue	1.98	1.82	1.53
necrotic tissue	2.08	2.04	1.72

Experiment 180: I to IV
Female rat born $^{11}/_{10}$ 1944, inoculated $^{24}/_1$ 1945

	Date	Weight in gm	Volume of sarcoma axes in cm
I.	$^{13}/_2$	156	$1.0 \times 1.6 \times 0.9$
	$^{20}/_2$	165	$0.8 \times 1.5 \times 1.0$
II.	$^{13}/_2$	139	$1.1 \times 2.2 \times 1.3$
	$^{20}/_2$	156	$0.8 \times 1.7 \times 1.1$
III.	$^{13}/_2$	165	$0.8 \times 1.5 \times 0.8$
	$^{20}/_2$	168	very small
IV.	$^{13}/_2$	150	
	$^{20}/_2$	149	very small

Activity of 1 mgm desoxyribosenucleic acid P, expressed in percentage
of the activity of 1 mgm inorganic P from the

sarcoma P	plasma P	liver P
2.76	1.61	1.10

Experiment 186: I to X
Male rat born January 1945, inoculated $^{10}/_4$

	Date	Weight in gm	Volume of sarcoma axes in cm
I.	$^{22}/_2$	215	$1.3 \times 2.4 \times 2.0$
	$^{24}/_2$		$1.3 \times 2.3 \times 2.0$
II.	$^{22}/_2$	210	$0.8 \times 1.4 \times 1.1$
	$^{24}/_2$		$0.7 \times 1.2 \times 1.1$
III.	$^8/_2$	148	$0.7 \times 1.3 \times 1.9$
	$^{22}/_2$		$0.7 \times 1.2 \times 0.9$
IV.	$^{22}/_1$	215	$0.6 \times 1.5 \times 0.8$
	$^{24}/_1$		$0.6 \times 1.4 \times 0.9$
V.	$^{22}/_1$	200	$1.1 \times 1.5 \times 1.2$
	$^{24}/_1$		$1.0 \times 1.4 \times 1.2$
VI.	$^{22}/_1$	173	$1.0 \times 1.7 \times 1.4$
	$^{24}/_1$		$1.0 \times 1.5 \times 1.4$
VII.	$^{22}/_1$	175	$1.4 \times 2.4 \times 2.0$
	$^{24}/_1$		$1.3 \times 2.3 \times 1.9$
VIII.	$^{22}/_1$	205	$0.7 \times 1.2 \times 1.0$
	$^{24}/_1$		$0.7 \times 1.1 \times 0.9$
IX.	$^{22}/_1$	193	$0.9 \times 1.2 \times 0.9$
	$^{24}/_1$		$0.8 \times 1.2 \times 0.9$
X.	$^{22}/_1$	194	$0.9 \times 1.3 \times 1.0$
	$^{24}/_1$		$0.9 \times 1.3 \times 1.0$

Activity of 1 mgm desoxyribosenucleic acid P, expressed in percentage of the activity of 1 mgm inorganic P from the

sarcoma	plasma	liver
1.12	1.05	1.28

Activity of 1 mgm desoxyribosenucleic acid P, expressed in percentage of the activity of 1 mgm inorganic P from the

sarcoma P	plasma P	liver P
2.57	1.18	0.94

Experiment 54: I to III.

Volumina of the sarcomata are stated in *K. Sv. Vet.-Akad. Arkiv f. Kemi A* **17** No. 30, 0. 41.

Activity of 1 mgm desoxyribosenucleic acid P, expressed in percentage of the activity of 1 mgm inorganic P from the

sarcoma	plasma	liver
1.19	1.05	1.03

In Table 2, data on the mean renewal rate of desoxyribosenucleic acid in 26 sarcomata are given.

(A) is the mean average for each separate experiment involving several animals.

(B) is the mean figure, when all the sarcomata are considered collectively.

TABLE 2

Activity of 1 mgm desoxyribonucleic acid P of *retrograde* Jensen sarcomata, expressed in percentage of the activity of 1 mgm inorganic P from the

	Sarcoma	Plasma	Liver
(A)	1.92	1.34	1.18
(B)	2.14	1.42	1.19

In Table 3 we state the ratio of the activity of 1 mgm desoxyribosenucleic acid P, to that of 1 mgm inorganic P from the sarcoma, the plasma and the liver respectively, as found earlier.

A comparison of the figures in Tables 2 and 3 leads to the conclusion that there is no pronounced difference between the percentages of newly formed desoxyribosenucleic acid in retrograde and in growing sarcomata; the difference amounting only to 12 per cent when considering the "sarcoma scale", and to 25 per cent when considering the "plasma scale". The significance of these scales is discussed below.

The autolysis of the tissue, followed by a regression of the sarcoma, leads to a decrease in the total desoxyribosenucleic acid content of the

TABLE 3

Activity of 1 mgm desoxyribosenucleic acid P of *growing* Jensen sarcomata in percentage of the activity of 1 mgm inorganic P from the

	Sarcoma	Plasma	Liver
(a) ⁽¹⁾	2.05	1.93	1.07
(b) ⁽²⁾	2.17	1.66	1.07
(c) ⁽³⁾	2.37	1.89	—

⁽¹⁾ H. EULER, and G. HEVESY, *Kgl. Danske Vidensk. Selskab, Biol. Medd.* of 7, 8 (1942).

⁽²⁾ H. EULER, and G. HEVESY, *Sv. Vet.-Akad. Arkiv f. Kemi*, A 17, No. 30 (1944).

⁽³⁾ Average of 4 not previously published results.

sarcoma, while the desoxyribosenucleic acid concentration in the sarcoma remains practically unchanged. We found the mean unpurified desoxyribosenucleic acid content of 1 gm growing sarcoma to be 9.2 mgm, this value representing the average of a very great number of investigated Jensen sarcomata. For the spontaneously retrograde sarcoma the corresponding figure was found to be 8.8 and for benzpyrene produced sarcoma (cf. p. 738) the value was 8.0

In the growing sarcoma an increase of the total desoxyribosenucleic acid content by 1 per cent during the experiment leads to a formation of labelled molecules up to at least 1 per cent of the desoxyribosenucleic acid content of the sarcoma. Such an increase is thus clearly indicated by the radioactive method. A decrease of the desoxyribosenucleic acid content by 1 per cent, however, will hardly be indicated by tracer experiments, since, to a very large extent, non-labelled molecules disappear. Should we find a decreased percentage new formation of desoxyribosenucleic acid in the retrograde sarcoma, this result would thus indicate that the mechanism of desoxyribosenucleic formation does not function normally in the retrograde sarcoma. As seen above, this is not the case; or the case only to a very restricted extent.

This result illustrates the fact that the tissue autolysis, followed by resorption, (the necrotic process) has very little in common with the more subtile enzyme "new formation" process; a result which is in no way surprising. TAUROG and his associates⁽¹⁾ placed surviving liver slices for a few hours in a Ringer solution containing labelled phosphate. An appreciable part of the ³²P added was found to be present at the end of the experiment in the phosphatides isolated in the tissue slices. A quantitative determination of the extent of renewal of the tissue phosphatide was not carried out, but it can be estimated to have amounted to a few per cent. Simultaneously the decrease in the total phosphatide content of the tissue slices was determined. It was found to be as much as 20

⁽¹⁾ A. TAUROG, J. L. CHAIKOFF and J. PERLMAN, *J. Biol. Chem.* **145**, 281 (1942).

per cent. Thus, with a substantial autolytic decomposition of the tissue phosphatides, a less pronounced formation of labelled phosphatides takes place. The latter more subtle process can be almost quantitatively suppressed by 0.01 mgm KCN, while the extent of autolysis is not at all diminished by the presence of cyanide; a slight decrease of the rate of autolysis being even observed.

In our experiments⁽²⁾ on the formation of labelled desoxyribosenucleic acid in surviving slices of Jensen sarcoma, incubated at 37° in a Ringer solution containing labelled phosphate, we found, in experiments taking 4 hours, about 0.1 per cent of the desoxyribosenucleic acid molecules present at the end of the experiment to be labelled. The desoxyribosenucleic acid content of such slices was found to be diminished by 25%, due to autolysis, in the course of 24 hours at 20°.

In a retrograde sarcoma the tissue not involved in the necrotic process is thus showing a almost normal desoxyribosenucleic acid formation. Since in the retrograde sarcoma the percentage formation of labelled desoxyribosenucleic acid is not appreciably lower than in the growing sarcoma, we have to conclude that a growth of the intact parts of the retrograde sarcoma takes place as well; possibly at a somewhat lower rate as indicated by the figures of Table 2.

In some of our earlier experiments we irradiated the rats all through the experiment. In these experiments the rats were still fixed to a table after the injection of the labelled phosphate had taken place. The ratio of the specific activity of the desoxyribosenucleic acid P, to that of the inorganic P of the sarcoma and the plasma respectively, was found in some of these experiments to be much lower than in our usual experiments, where the rats were fixed to a table during the irradiation, but not after the injection of the labelled phosphate had taken place. The low radioactivity found when the rats were fixed to a table, and irradiated all through the experiment, was discovered not to be due to a correspondingly diminished turnover of the desoxyribosenucleic acid under the action of radiation, but mainly to a disturbance in the circulation of the rat. We found, not in all, but in several of the control experiments in which rats were fixed to a table without being irradiated, very low activity figures for the desoxyribosenucleic acid. The disturbed circulation results in a low ratio of the specific activity of the inorganic P of the plasma and the sarcoma, which can become in such experiments with disturbed circulation as low as $\frac{1}{20}$. In such cases a very appreciable part of the inorganic P of the sarcoma is made up of extracellular inorganic P. Furthermore, the disturbed circulation also influences the resorption of the injected phosphate, and the difference between the

⁽²⁾ L. AHLSTRÖM, H. EULER and G. HEVESY, *Sv. Vet-Akad. Arkiv f. Kemi, A* **21**, No. 6 (1945).

end and the mean value of the specific activity of the tissue inorganic P during the experiment becomes very appreciable.

That the fixing of the rat on to the table all through the experiment does not necessarily interfere with the normal circulation, is seen from the following activity ratios:

	Percentage ratio of the specific activity of the nucleic acid P to that of the inorganic sarcoma P	Percentage ratio of the specific activity of the nucleic acid P to that of the inorganic plasma P
Control	2.08	1.47
Fixed to table	2.08	1.54

In this case the muscles of the rat presumably relaxed. That the effect of fixing can obstruct the circulation to a high degree, is seen from the following figures:

Fixed to table 0.234 0.111

In our later experiments, in which the animal was irradiated all through the experiment, we placed the rat in a beaker coated with black paper. The X-ray dose administered was controlled by placing a micro-ionising chamber on the sarcoma. In these experiments no appreciable difference was found between the results obtained either when the irradiation took place only before the injection or when it took place all through the experiment; as seen in Table 4.

TABLE 4. IRRADIATION OF RATS IN GLASS BEAKER WITH A TOTAL DOSE OF 1000 R

	Control	Irradiated before injection only	Irradiated all through the experiment
Specific activity of desoxyribosenucleic acid P, expressed in percentage of the specific activity of sarcoma inorganic P	2.95	0.983	0.986 1.17 1.08
Specific activity of desoxyribosenucleic acid P, expressed in percentage of the specific activity of plasma inorganic P	2.29	0.775	1.02 — 1.15

Desoxyribosenucleic Acid Formation in Benzpyrene Sarcomata

In a few cases we determined the rate of renewal of desoxyribosenucleic acid in sarcomata obtained by grafting tumour tissue produced by treatment of rats with benzpyrene. The results obtained are seen in Table 5.

TABLE 5.
PERCENTAGE NEW FORMATION OF DESOXYRIBOSENUCLEIC ACID
IN BENZPYRENE SARCOMATA

No. of experiment	Weight of sarcoma in gm	Activity of 1 mgm nucleic acid P expressed in percentage of the activity of 1 mgm inorganic P from the		
		sarcoma	plasma	liver
163 : 1	4	1.71	1.91	1.20
163 : 2	2			
172 : a	50	1.44	1.17	0.95
172 : b	5	1.27	1.05	0.85
174 : a	12	1.38	0.81	0.60
	3.4			
174 : b	3	1.20	0.90	0.93
	6			
179	10	2.05	1.81	1.18
	9			
Mean value		1.51	1.24	0.97

TABLE 6. — PERCENTAGE NEW FORMATION OF DESOXYRIBOSENUCLEIC ACID IN RETROGRADE BENZPYRENE SARCOMATA

No. of experiment	Date of inoculation 1944	Weight of rating	Sarcoma volume; axes in cm
163 : 1	24/8	125	
	22/9	153	$2.0 \times 3.9 \times 1.5$
	3/10	193	$1.4 \times 3.1 \times 1.5$
	9/10		$1.3 \times 2.4 \times 1.3$
163 : 2	8/9	180	$1.2 \times 2.4 \times 1.3$
	9/9		$1.0 \times 1.8 \times 1.3$

Activity of 1 mgm nucleic acid P expressed in percentage of the activity of 1 mgm inorganic P from the

sarcoma	plasma	liver
1.71	1.91	1.20

Activity of 1 mgm nucleic acid P expressed in percentage of the activity of 1 mgm inorganic P from the

sarcoma	plasma	liver
0.99	0.81	0.66

The mean new formation figure obtained for the benzpyrene sarcomata is lower than the corresponding figure obtained for Jensen sarcomata. While we investigated several hundred Jensen sarcomata, the number

TABLE 6. (CONT)

No. of experiment	Date of inoculation 1944—1945	Weight of rating	Sarcoma volume; axes in cm
178 : 1	15/12	147	
	10/1	166	$1.75 \times 3.2 \times 2.0$
	23/1	175	$1.4 \times 3.1 \times 1.7$
	30/1	175	$1.3 \times 2.8 \times 1.4$
	7/2	210	$1.2 \times 2.4 \times 1.0$
	9/2		$1.1 \times 2.4 \times 1.0$
178 : 2	30/1	200	$1.3 \times 2.2 \times 1.3$
	7/2	230	$1.3 \times 1.9 \times 1.4$
	9/2		$1.3 \times 1.8 \times 1.3$
178 : 3	30/1	212	$1.7 \times 4.9 \times 2.6$
	7/2	240	$1.6 \times 4.6 \times 1.7$
	9/2		$1.5 \times 4.0 \times 1.7$

of benzpyrene sarcomata investigated amounts to ten only; the difference obtained in the renewal rate of the two types of sarcoma is therefore to be interpreted cautiously.

We investigated furthermore 5 retrograde benzpyrene sarcomata. Two (163:1 and 163:2) are showing almost normal turnover rates, while appreciably lower values were found for the remaining 3 sarcomata.

Desoxyribosenucleic Acid Formation in Sarcomata of Colchicine Treated Rats

In view of the effect of colchicine on the mitotic process, it was of interest to investigate the effect of colchicine treatment on the turnover of desoxyribosenucleic acid, and to determine if it is possible to obtain similar effects by colchicine treatment as obtained by irradiation. The following experiments were carried out:

(a) Rats weighing 138 and 111 gm respectively were injected with 50 γ colchicine. Injection was repeated after a lapse of 6 days, and one day later labelled sodiumphosphate was administered by subcutaneous injection. The rat was killed, as in all our other experiments, 2 hours later, and the desoxyribosenucleic acid of the sarcoma, weighing 23 gm, and the inorganic P of sarcoma and plasma were isolated.

(b) In these experiments 50 γ colchicin ewere injected subcutaneously, one day before the administration of ^{32}P to rats weighing 112, 126, 165 and 125 gm respectively.

(c) In this experiment 150 γ colchicine were administered on three consecutive days. One day later ^{32}P was injected.

(d) ^{32}P was administered 3 days after injecting 50 γ colchicine. Weight of rats was 120 and 155 gm resp. Weight of the only slightly necrotic sarcoma was 9 and 7 gm respectively.

TABLE 7. — COLCHICINE TREATED RATS

Specific Activity of Desoxyribosenucleic Acid expressed in Percentage of the Specific Activity of the Inorganic P from the sarcomata and plasma:

Experiment (particulars see above)	Sarcoma	Plasma
(a)	1.78	1.68
(b) I	2.69	1.94
II	2.01	1.94
III	1.52	1.47
IV	1.06	1.14
(c)	1.63	2.10
(d) I	1.46	1.44
II	1.52	1.98
(e)	1.05	0.99
Mean value	1.66	1.62

(e) 50 γ colchicine were administered both 7 days before, and 3 days before the injection of labelled phosphate. Weight of rats was 97 and 135 gm resp. at the start, 105 and 150 gm at the end of the experiment. Weight of sarcomata 5 and 4 gm resp. with hard necrotic inclusions.

The results obtained are seen in Table 7.

The mean value obtained for the extent of renewal of desoxyribose-nucleic acid in the colchicine treated rats is about $\frac{1}{5}$ lower than the corresponding value for the controls (cf. Table 3); the individual values vary greatly. The variation in the "plasma scale" is less than it is in the "sarcoma scale", thus indicating a somewhat enhanced phosphate permeability of the sarcomata in colchicine treated rats.

In some of our experiments, we studied the combined effect of Roentgen radiation colchicine injection on nucleic acid formation. Table 8 denotes an experiment in which

(a) Colchicine was administrated 1 day before the injection of ^{32}P .

(b) Colchicine was administered; the following day the rat was irradiated with 30 r per minute for 10 minutes, ^{32}P then injected and the rat killed 2 hours later.

(c) The same procedure was followed as in (b), but without colchicine treatment.

(d) The experimental conditions were those of (b) with the sole exception that the rat was irradiated with only 15 r per minute, for 10 min. The results obtained were seen in Table 8.

The combined effect of colchicine and X-rays is, as shown by the above figures, no greater than is the effect of X-rays alone in influencing the turnover rate of desoxyribosenucleic acid of the sarcoma. In view

of the results obtained by LEVINE,⁽¹⁾ who found a combination of X-ray and colchicine treatment of roottips to be very effective in retarding the growth of *Allium Cepa*, a more detailed investigation of such a combined effect on the nucleic acid turnover in animal tissues may be of interest.

TABLE 8. — PERCENTAGE NEW FORMATION OF
DESOXYRIBOSENUCLEIC ACID IN COLCHICINE
TREATED AND IRRADIATED RATS

	Percentage Specific Act- ivity of Nucleic Acid P: Specific Activity of in- organic sarcoma P	Percentage Specific Act- ivity of Nucleic Acid P: Specific Activity of in- organic plasma P
(a)	1.85	1.36
(b)	1.43	1.25
(c)	1.43	1.53
(d)	1.61	1.61

Replacement of Irradiation with Roentgen Rays by Radiation Emitted by Injected Radio-Elements

By injecting very substantial amounts of ^{32}P we can expect to find a reduced formation of labelled desoxyribosenucleic acid. 1 microcurie per gm tissue produces in the course of 2 hours an ionisation corresponding to 3.5 r units⁽²⁾. To produce 175 r units in the course of 2 hours we have to administer about 50 microcuries per gm, or 7.5 millicuries, to a rat weighing 150 gm. In this calculation the excretion of some of ^{32}P administered during the experiment taking 2 hours is disregarded.

We have not at our disposal sufficiently strong radioactive preparations to carry out such an experiment. In one experiment, however, we injected 3.9 millicuries of radiosodium 5 hours before administering a tracer dose of ^{32}P . The mean radiosodium activity per gm tissue of the rat weighing 190 gm was in this experiment 20.5 microcurie. The β -rays of the radiosodium acted upon the tissue for $5 + 2 = 7$ hours producing an ionisation corresponding to 250 r units. Radiosodium emits, beside β -rays, also γ -rays, which are only partly absorbed into the rat's body. The ionisation produced by these rays in the rat can be estimated as corresponding roughly to a total dose of about 60 r in the course of 7 hours, bringing the total dose up to about 310 r.

The radiosodium was administered by subcutaneous injection in the form of 1.2 ml physiological sodiumchloride solution. In the control experiment the same volume of non-radioactive sodiumchloride was

(1) M. LEVINE, *Cancer Res.* **3**, 107 (1943).

(2) L. D. MARINELLI, *Amer. J. Roentgenol.* **47**, 210 (1942).

injected 5 hours before the administration of a tracer dose of ^{32}P . The results obtained are seen in Table 9.

TABLE 9. — EFFECT OF ADMINISTRATION OF RADIOSODIUM ON THE FORMATION OF LABELLED DESOXYRIBOSENUCLEIC ACID IN THE LIVER IN THE COURSE OF 2 HR.

Specific Activity of Nucleic Acid P expressed in Percentage of the Activity of 1 mgm inorganic P from

	Liver	Plasma
Control	0.125	0.121
^{24}Na injected	0.058	0.071

In this experiment, as seen above, the radiation emitted by radiosodium was effective in diminishing the formation rate in the liver of the desoxyribosenucleic acid.

In experiments of short duration the replacement of Roentgen radiation by radiation emitted by the injected radioactive substances has no outstanding advantage. In experiments of long duration, however, the last mentioned technique is often much to be preferred.

Summary

The percentage new formation of desoxyribonucleic acid phosphorus is only slightly smaller in the spontaneously regressive Jensen sarcoma of the rat than in the growing tumour. This fact indicates that the enzyme mechanism, responsible for the incorporation of phosphate into the desoxyribonucleic acid molecule, is hardly disturbed in the regressive sarcoma.

Treatment of rats with colchicine influences the rate of formation of desoxyribosenucleic acid phosphorus to a minor extent.

A somewhat lower formation rate of desoxyribosenucleic acid phosphorus is observed in grafted benzpyrene tumours, than in Jensen sarcoma.

The rate of formation of labelled desoxyribosenucleic acid under uninterrupted irradiation during the whole experiment, previously reported to be very low, is to be explained by a disturbance in the circulation of the rat, and not by a correspondingly low rate of formation of labelled desoxyribosenucleic acid.

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74. THE INDIRECT EFFECT OF X-RAYS ON THE JENSEN-SARCOMA

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SEVERAL observations have been made that besides the direct effect of X-rays there is also an indirect effect which is able to influence the division of cells. KOK and VORLAENDER⁽¹⁾ observed, for example, an indirect effect in the "unirradiated" skin of the mouse, in addition to the direct radiation effect. This "indirect" effect is fundamentally similar to the direct one, yet a more or less important graded difference can be recognized which always points to weakening.

In another communication KOK stated that he had similar good results with a 25 per cent dosage delivered to the whole body of the animal as with optimum local irradiation.

RUSS, CHAMBERS and SCOTT⁽²⁾ had already found, a long time ago, that Jensen-sarcoma transferred by inoculation into rats grew more slowly in those which had been repeatedly exposed to small doses of irradiation than in the unirradiated controls. These authors have shown⁽³⁾ more recently that the inoculated Jensen-sarcomas grow better in a normal tissue than in tissue whose surroundings have been irradiated. These experiments have been continued in recent years⁽⁴⁾. RUSS and SCOTT compare the growth of sarcomas of approximately the same size from two rats of the same age. In one case, only the sarcoma was irradiated, while the whole of the body was protected by lead sheet from the effect of radiation. In the other case, they irradiated only the surroundings of the sarcoma, while the sarcoma itself and the remainder of the body were protected by means of lead.

Some time ago we showed, with the help of radioactive phosphorus as a tracer⁽⁵⁾, that the rate of synthesis of desoxyribose nucleic acid molecules

⁽¹⁾ KOK and K. VORLAENDER, *Strahlentherapie* **14**, 497 (1923); Fr. KOK, *Ibid* **18**, 90 (1924).

⁽²⁾ W. RUSS, CHAMBERS and G. M. SCOTT, *Proc. Roy. Soc. B* **92** (1921).

⁽³⁾ S. RUSS and G. M. SCOTT, *Brit. J. Radiol.* **32**, 289 (1927).

⁽⁴⁾ Cf. Medical uses of radium, *Brit. Soc. Radiol. N. S.* **14**, 9 (1942).

⁽⁵⁾ H. EULER and G. HEVESY, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **17**, 8 (1942); *Ark. Kem.* **17**, No. 30 (1944).

in a sarcoma is depressed by the action of X-rays, and we have used this method for studying the question of whether and to what extent a sarcoma protected against the direct effect of radiation is affected by irradiating other parts of the body. For this purpose we inoculated rats with two sarcomas, the one sarcoma being irradiated and the other being protected against the effect of X-rays by a covering of lead. Radioactive sodium phosphate was injected after irradiation. Two hours later the rats were killed and the deoxyribonucleic acid was isolated from both sarcomas. Comparison of the radioactivity of the two fractions with each other and with the activity of nucleic acid samples which had been obtained from the sarcomas of unirradiated controls permits answers to be given to the questions formulated above.

EXPERIMENTAL

In the first series of experiments rats were used in which both sarcomas were growing on the left and right of the back. The shortest distance between the sarcomas was about 4 cm. One sarcoma was protected against the effect of irradiation with a lead sheet at least 5 mm thick.

TABLE 1. — FORMATION OF NUCLEIC ACID IN UNIRRADIATED SARCOMAS.
THE OTHER SARCOMA IRRADIATED WITH 280—2000 r

Expt.	Weight of sarcoma (gm)	Dose ⁽¹⁾ (r)	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of		
			Liver P	Plasma P	Sarcoma P
105	12.5	1500	1.84	—	—
106	39	1500	0.094	0.25	0.21
107	36	1500	0.53	0.72	3.42
111: 2 ²	42	1000	0.40	—	0.99
112: 2	32.5	1200	0.39	0.39	0.79
114	15	1000	0.043	0.01	0.28
116	11.5	1000	1.26	0.79	1.46
117	18.5	950	0.83	0.86	0.33
118	20	280	0.93	1.11	1.20
120	12	280	0.83	0.83	1.20
122: 1	31	1200	0.42	0.52	0.34
122: 2 ³	29	1260	0.26	0.38	0.39
128: 1	7	700	0.60	1.28	1.18
130: 2	15.0	2000	0.71	0.70	0.88
131: 1	9.0	2000	1.00	0.64	0.67
131: 2	6.0	2000	0.27	1.95	2.07
134: 1	26.0	2000	0.55	0.50	0.68
Mean value			0.67	0.52	1.00

TABLE 2. — FORMATION OF NUCLEIC ACID IN SARCOMAS IRRADIATED WITH 280—2000 r

Expt.	Weight of Sarcoma (gm)	Activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of		
		Liver P	Plasma P	Sarcoma P
105	13.0	1.33	—	—
106	40.0	0.15	0.39	0.15
107	30	0.42	0.57	2.95
111: 2	30	0.71	—	1.27
112: 2	25.5	0.38	0.38	0.51
114	14	0.04	0.012	0.205
116	6	2.18	1.35	1.88
117	13	0.65	0.67	0.78
118	13.5	0.87	1.03	1.08
120	8	0.51	0.51	0.51
122: 1	13	0.58	0.74	0.68
122: 2	22	0.64	0.94	0.68
126: 1	12	—	1.06	—
126: 2	14	—	0.41	0.51
130: 2	17.0	0.45	0.51	0.53
131: 1	8.0	0.40	0.76	0.82
131: 2	24.0	0.28	0.44	0.55
134: 1	16	0.43	0.40	0.54
Mean value	15	0.63	0.517	0.85

TABLE 3. — FORMATION OF NUCLEIC ACID IN UNIRRADIATED SARCOMA (ONLY SARCOMAS LYING FAR APART ARE CONSIDERED HERE)

Expt.	Activity of 1 mgm nucleic acid P as a percentage of the activity of		
	Liver P	Plasma P	Sarcoma P
117	0.83	0.86	0.33
118	0.93	1.11	1.20
120	0.83	0.83	1.20
122: 1	0.42	0.52	0.34
122: 2	0.26	0.38	0.30
126: 1	—	0.67	—
126: 2	—	1.28	1.18
130: 2	0.71	0.64	0.67
131: 1	1.00	1.95	2.07
131: 2	0.27	0.42	0.97
134: 1	0.55	0.50	0.68
Mean value	0.71	0.83	0.89

while the other sarcoma was irradiated with doses from 280 to 2000 r. The time of irradiation amounted to 3 to 28 min and the tube voltage was 165 kV. A 0.5 mm copper foil and a 1 mm aluminium foil were used as radiation filters. In the later experiments the sarcomas were placed as far as possible from each other. One sarcoma was situated on the right side behind the neck, the other on the left side near the tail. The

TABLE 4. — FORMATION OF NUCLEIC ACID IN IRRADIATED SARCOMA (ONLY SARCOMAS LYING FAR APART ARE CONSIDERED HERE)

Expt.	Weight of sarcoma (gm)	Activity of 1 mgm of nucleic acid as a percentage of the activity in 1 mgm of		
		Liver P	Plasma P	Sarcoma P
117	13	0.65	0.67	0.78
118	13.5	0.87	1.03	1.08
120	8	0.51	0.51	0.51
122: 1	13	0.58	0.74	0.68
122: 2	22	0.64	0.94	0.68
126: 1	12.0	—	1.06	—
126: 2	14.0	—	0.41	0.51
128: 1	10	0.64	0.75	0.70
130: 2	17.0	0.45	0.51	0.53
131: 1	8.0	0.40	0.76	0.82
131: 2	24.0	0.28	0.44	0.54
134: 1	16	0.43	0.40	0.54
Mean value		0.55	0.68	0.67

(¹) Irradiation dose of the other sarcoma

(²) Injected 11 days after irradiation.

(³) Injected 6 days after the last irradiation.

shortest distance between the boundaries of the sarcomas amounted to about 7.5 cm. In one experiment, the right-hand sarcoma was irradiated and the left-hand one protected. In the next experiment, the order was the reverse. The radiation dose received by the protected sarcoma was quite small, as was shown by the control with the Sieverts measuring device. In experiment 117, for example, in which one sarcoma was protected with an 8.5 mm thick lead sheet, measurement with the aid of a celluloid measuring chamber showed that the lateral total dose received was 6 r for the right-hand and 4 r for the left-hand one, while the surface of the sarcoma was irradiated with a total of 12 r. A measuring chamber indicated that the directly irradiated sarcoma received a dose of 980 r in the same time.

TABLE 5. — FORMATION OF NUCLEIC ACID IN DOUBLE SARCOMAS OF UNIRRADIATED RATS

Expt.	Weight of sarcoma (g)		Activity of 1 mgm of nucleic acid P as a percentage of 1 mgm of		
			Liver P	Plasma P	Sarcoma P
110: 1	right	14	0.60	1.33	1.90
	left	33	1.19	2.65	5.20
110: 2	right	35.5	0.88	0.76	0.69
	left	31.0	0.83	0.71	0.91
127: 1	in front	10.0	0.76	0.62	0.70
127: 1	behind	10.0	0.81	0.66	1.17
127: 2	in front	7.0	1.75	1.86	—
127: 2	behind	2.0	2.86	3.01	3.84
128: 2	in front	6.0	1.71	2.26	2.27
128: 2	behind	6.0	1.33	2.25	2.34
132: 1	in front	29.0	1.15	1.35	2.01
	behind	14.0	0.75	0.87	1.24
132: 2	in front	21.0	1.29	1.37	1.90
	behind	12.0	1.35	1.43	2.06
133: 1	in front	15	0.72	1.01	0.89
	behind	9	0.76	1.06	1.22
Mean value		14.6	1.20	1.44	1.89

TABLE 6. — AVERAGE VALUES FOR THE FORMATION OF NUCLEIC ACID IN IRRADIATED AND UNIRRADIATED DOUBLE SARCOMAS (EXPERIMENTAL TIME, 2 hr)

Activity of 1 mgm of nucleic acid P as a percentage of 1 mgm of	Irradiated	Unirradiated	Unirradiated rats (controls)
Liver P	0.63	0.67	1.20
Plasma P	0.57	0.52	1.44
Sarcoma P	0.85	1.00	1.89
<i>For sarcomas which are far apart</i>			
Liver P	0.55	0.71	
Plasma	0.68	0.83	
Sarcoma P	0.67	0.89	

In one case, a metal measuring chamber was placed in the centre of the sarcoma in a narcotized rat and the dose received by the lead-shielded sarcoma was measured while the other unprotected sarcoma was irradiated with 2000 r. The measuring chamber indicated a total dose of

TABLE 8. — VOLUME MEASUREMENTS ON DOUBLE SARCOMAS

(Weight of rats about 150 gm Male. Inoculated 3 May 1944. Each was irradiated with 500 r on 17, 20 and 22 May. a = anterior sarcoma, b = posterior sarcoma)

Rat	Date	Volume of sarcoma, cm ³	Daily percentage volume increase	Mean value of the daily percentage volume increase, before, during and after irradiation	Ratio of the volume increases before and during or before and after irradiation
1: a irrad.	15/5	1.13			
	16	1.68	48.7	48.7	—
	17	2.48	47.6		
	19	3.61	45.6		
	20	3.95	9.4	13.3	3.6
	22	4.41	11.6		
	24	5.67	28.6		
	26	8.86	56.3	14.2	3.4
	30	10.54	19.0		
	2/6	16.04	52.2		
	5	20.08	25.2	11.7	4.1
	7	18.56	—7.6		
	10	19.36	4.3		
	12	21.42	10.6	1.0	4.8
1: b prot.	16/5	0.17			
	17	0.25	47.1	47.1	—
	19	0.34	36.0		
	20	0.42	23.5		
	22	0.50	19.0	15.7	3.7
	24	0.41	42.0		
	26	1.43	101.4		
	30	2.02	41.3	24.9	1.9
	2/6	2.23	10.4		
	5	5.17	131.8		
	7	5.88	13.7	21.8	2.2
	10	7.39	25.7		
	12	7.85	62.2		
				14.5	3.2

13 r. The intensity of the scattered radiation received by the protected sarcoma during the irradiation of the unprotected sarcoma thus amounted to only about $\frac{3}{4}$ per cent of the radiation dose supplied to the unprotected sarcoma. Immediately after irradiation 0.1 cm³ of sodium phosphate solution (pH 8) containing 0.3 mgm P and an activity of about 3 μ c was injected subcutaneously. Two hours later the rat was killed and the deoxyribonucleic acid of the two sarcomas and the "free" P of the two sarcomas, the plasma and the liver were isolated. Comparison of the ratio of the activity in 1 mgm of nucleic acid P and in 1 mgm of free sarcoma P is a measure of the newly formed nucleic acid during the experimental time of 2 hr. Comparison of the activity of the nucleic acid P with that of the plasma P or of the liver P can be used as a control.

TABLE 7. — FORMATION OF NUCLEIC ACID IN DOUBLE SARCOMAS (TIME OF EXPERIMENT, $\frac{1}{2}$ hr; DOSE 1500 r)

Expt.	Weight of sarcoma (g)	Activity of 1 mgm of nucleic acid P as a percentage of 1 mgm of		
		Liver P	Plasma P	Sarcoma P
(a) <i>Unirradiated sarcomas</i>				
108: 1	17	0.37	0.23	0.96
108: 2	27	0.105	0.058	0.326
109	41	0.042	0.098	0.071
(b) <i>Irradiated sarcomas</i>				
108: 1	14	—	—	—
108: 2	24	0.080	0.044	0.332
109	27.5	0.040	0.110	—

RESULTS

In Table 1 are recorded the experimental results which were obtained in the study of one sarcoma protected against the effects of radiation, while Table 2 contains the corresponding data for the irradiated sarcomas. Tables 3 and 4 contain the experimental results relating only to the sarcomas which were far removed from each other (one near the neck and the other near the tail). As a check, we have also investigated the rate of formation of labelled desoxyribonucleic acid in double sarcomas of unirradiated rats. The result of this experiment is seen in Table 5, while Table 6 contains a summary of all results.

By taking into account the studies of all the sarcomas in irradiated rats it is found that the direct irradiation reduces the nucleic acid formation to 45 per cent of that taking place in the sarcomas in the control animals, while the metabolism in the shielded sarcoma is only reduced to 53 per cent. When only the widely separated sarcomas are considered, the corresponding values are 35 and 47 per cent.

From these results it follows that the synthesis of nucleic acid in these sarcomas, which have not been exposed to X-rays, is also subject to the action of X-rays, but the effect is somewhat less and is only about four-fifths of that found in the study of the irradiated sarcoma. These results correspond to the events occurring in the sarcoma within 2 hr of the irradiation.

We have also performed a few experiments in which the rats were killed only $\frac{1}{2}$ hr after injection of the radioactively labelled phosphate solution. In so far as conclusions can be drawn from the very few values contained in Table 8, the nucleic acid formation is not very different in the shielded and irradiated sarcomas in this $\frac{1}{2}$ hr experiment also.

TABLE 9. — COLLECTION OF RESULTS OF MEASUREMENTS OF THE VOLUME INCREASE IN IRRADIATED AND SHIELDED SARCOMAS (One of the two sarcomas was irradiated, the other being shielded during the irradiation)

No.	Mean daily percentage increase in volume				
	Before	During	After the irradiation, periods		
			1 8 days	2 6 days	3 7 days
A) Irradiated sarcomas					
1: A	48.2	13.3	14.2	11.7	1.0
2: A	63.5	17.5	7.5	7.7	
3: A	32.8	22.6	11.8	4.9	
4: A	61.5	— 0.2	— 4.1	— 17.5	
5: A	35.7	15.6	2.0	0.1	
6: A	47.5	4.3	14.1	21.4	
7: A	27.5	22.9	5.2	— 1.2	
8: A	47.3	13.4	2.3	11.5	— 5.2
9: B	59.4	21.5	5.1		7.7
10: B	21.8	18.7	10.8	— 6.5	
11: B	71.2	25.0	— 10.6	— 3.8	
12: B	26.5	24.3	11.3	2.4	
13: B	15.4	32.8	10.1	1.4	
14: B	35.0	22.2	9.4	0.9	
15: B	76.9	23.9	5.8	7.9	
	44.7	18.5	6.3	2.9	1.2
B) Shielded sarcomas					
1: B	47.1	15.7	24.9	21.8	14.5
2: B	39.6	18.2	14.1	14.5	
3: B	45.2	18.0	21.6	8.8	
4: B	21.2	24.2	18.3	13.6	14.9
5: B	51.4	31.2	15.3	4.6	
6: B	59.5	27.2	20.7	12.8	
7: B	77.4	19.8	12.7	3.5	
8: B	42.4	30.6	26.7	13.0	— 1.5
9: A	62.5	20.1	4.1		
10: A	55.3	26.5	11.3	6.7	— 0.4
11: A	44.8	28.9	17.9	22.9	
12: A	31.2	22.8	12.4	3.2	
13: A	20.6	23.7	21.9	7.2	
14: A	44.1	17.9	16.3	5.2	
15: A	41.5	38.0	21.0	3.2	
	45.6	24.2	17.3	10.1	6.9

Effect of Irradiation on the Increase in Volume of Irradiated and Shielded Sarcomas

As we have found, irradiation inhibits the formation of nucleic acid in the 2 hrs following the exposure almost to the same extent in the shielded as in the irradiated sarcomas. On an earlier occasion⁽¹⁾ we found that in most cases the effect of X-rays which inhibits the formation of nucleic acid decreases with time. The question therefore arose as to how the effects of the X-rays on the shielded sarcoma and the unprotected tumour are related at longer times, after irradiation has taken place.

We have tried to answer this question by following the increase in volume of the irradiated and shielded sarcomas. Some double sarcomas which were inoculated in parallel into the two halves of the back (cf. p. 755) and others, of which one was placed behind the neck and the second near the tail on the right and left, respectively, were studied in this experiment. The volume increase was measured for a few days before, then for 4–6 days during irradiation and finally once every day after completion of the irradiation. In order to permit sufficiently accurate

TABLE 10. — VOLUME MEASUREMENTS ON IRRADIATED AND SHIELDED SARCOMAS

(Weight of rats about 150 gm Female. Inoculated on 3 November 1943.

Irradiated on each second day normally with a total of 500 r each;

a = anterior sarcoma, b = posterior sarcoma)

Rat	Date	Volume of sarcoma (cm ³)	Daily percentage volume increase	Mean value of the daily percentage volume increase, before, during and after irradiation
4a unirrad.	15/11	2.06		
	16	3.95	91.7	36.9
	18	4.70	19.0	
	20	7.39	57.2	
	22	11.42	54.5	22.6
	24	14.11	23.6	
	26	18.35	30.0	7.5
	29	19.74	7.6	
4b irrad.	15/11	1.76		
	16	2.90	64.8	32.2
	18	3.82	31.7	
	20	4.54	18.8	
	22	6.47	42.5	12.7
	24	7.43	14.8	
	26	6.97	— 6.2	— 2.4
	29	6.55	— 6.0	

⁽¹⁾ H. EULER and G. HEVESY, *Ark. Kem.* **17 A**, No. 30 (1944).

measurement of the volume of the sarcomas, the volume must amount to 1 cm³ or more. Such sarcomas exhibit a smaller increase in volume in the later phases of the experiment than in the earlier ones. Table 8 shows the results of volume measurements which were performed on two sarcomas placed at a far distance (8 cm) from each other. It is evident from the table that the two sarcomas exhibit very nearly the

TABLE 11. — SUMMARY OF THE RESULTS OF VOLUME MEASUREMENTS ON IRRADIATED AND UNIRRADIATED SARCOMAS (ONE OF THE TWO SARCOMAS WAS IRRADIATED, THE OTHER SHIELDED)

No.	Mean daily percentage increase in volume		
	Before	During	After irradiation
(A) <i>Irradiated sarcomas</i>			
1 b	27.6	22.8	2.6
2 b	46.2	17.1	7.7
3 b	7.2	17.4	8.2
4 b	32.2	12.7	— 2.4
5 a	39.1	25.7	— 1.5
6 a	44.2	16.4	0.3
7 a	44.6	20.3	6.5
8 a	19.5	12.1	5.8
	32.6	18.1	3.4
(B) <i>Unirradiated sarcomas (shielded)</i>			
1 a	18.8	25.4	10.4
2 a	35.0	26.9	2.4
3 a	19.3	29.2	16.8
4 a	36.9	22.6	7.5
5 b	21.1	20.1	2.9
6 b	53.3	16.5	16.4
7 b	22.2	26.0	12.7
8 b	36.9	0.0	8.0
	30.4	20.8	9.6

same behaviour during the irradiation time. In the course of the first 8 days after irradiation and also in the next 6 days, the irradiated sarcoma grows at only about one-half the rate of the shielded one. Since the accuracy of measuring the volume of the sarcoma is limited, we base our conclusions not on a single value for the increase but on the average of the volume measurements made in a period of time (e.g. 6 days). The collected results from fifteen similar experiments is seen in Table 9.

It is evident from Table 9 that the volume increase of the shielded sarcoma during the irradiation period is not substantially different

from that of the unshielded one (ratio 1.3). In the 8 subsequent days, on the contrary, as also in the next 6 days, the ratio is nearly equal to 3. A still greater ratio is obtained by comparing the values for the third (7 day) period of the experiment. In the last time interval, however, only a few measurement are available since volume measurements during this time were either impracticable or insufficiently accurate.

TABLE 12. — COMPARISON OF THE INCREASES IN VOLUME OF THE TWO SARCOMAS IN UNIRRADIATED RATS

(Inoculated 3 November, 1943; a-anterior sarcoma, b-posterior sarcoma)

Rat	Date	Volume of sarcoma (cm ³)	Daily percentage increase in volume	Mean value of the daily percentage increase in volume
10a	15/11	2.02		
	16	2.90	43.6	31.4
	18	4.37	50.7	
	20	5.17	18.3	
	22	11.30	118.6	24.6
	24	12.47	10.4	
	26	18.06	44.8	
	29	20.58	14.0	11.6
	2/12	27.51	33.7	
10b	15/11	0.50		
	16	0.71	42.0	36.1
	18	1.18	66.2	
	20	2.60	120.3	
	22	2.90	11.5	27.5
	24	3.86	33.1	
	26	4.20	8.8	
	29	6.47	54.0	14.7
	2/12	10.00	54.6	

The results of another series of experiments are shown in Tables 10 and 11. The sequence of one experiment is recorded as an example in Table 10. Table 11 contains the summary of eight experimental sequences.

If we consider, on the other hand, the average value of the eight experiments recorded in Table 11 the increase during irradiation in the irradiated sarcoma is indeed only slightly less than in the unirradiated one, but during the following 5—6 days the volume increase in the irradiated sarcoma amounts only to about one-third of that occurring in the unirradiated growth. Therefore, we find the same result as in the case of the experimental series at first discussed.

Comparison of the Volume Increases in the Anterior and Posterior Sarcomas

In connexion with the study of the behaviour of irradiated and shielded sarcomas it was of interest to compare the increases in volume of the two unirradiated sarcomas placed by inoculation into the rat. To facilitate comparison of the results of this experiment with those of the

TABLE 13. — MEASUREMENTS OF VOLUME OF THE UNIRRADIATED POSTERIOR AND ANTERIOR SARCOMAS OF RATS. SUMMARY OF RESULTS

(Percentage increase calculated in the same way as for irradiated animals)

No.	3-day period	Subsequent 6-day period	Subsequent 8-day period
<i>Anterior sarcoma</i>			
9 a	45.0	22.6	11.6
10 a	31.4	24.6	10.8
11 a	37.5	18.1	10.6
12 a	34.2	19.9	14.0
13 a	20.4	28.3	— 8.6
14 a	12.9	— 5.0	— 8.6
15 a	35.8	31.4	7.3
16 a	7.9	8.6	—13.2
	28.1	18.6	4.6
<i>Posterior sarcoma</i>			
9 b	70.9	22.9	
10 b	36.1	27.5	14.7
11 b	24.8	22.0	6.5
12 b	34.1	11.0	11.3
13 b	—	32.2	18.4
14 b	9.5	13.5	—12.6
15 b	9.7	28.5	8.9
16 b	0.0	— 8.5	—30.9
	26.4	18.6	2.3

irradiation experiments we have determined the daily average increase in volume in three consecutive time intervals, i.e. in periods of 3, 6 and 8 days. Table 12 contains the example of an experimental sequence.

There is no essential difference between the increases in the two sarcomas. An average of the mean values for the eight experiments, which are summarized in Table 13, shows, on the contrary, that in the time interval corresponding to the irradiation period in the irradiation experiments, the percentage increases in the two sarcomas are nearly identical, but

that this does not hold good in the subsequent period in which the anterior sarcoma grows twice as quickly as the posterior sarcoma. This observation, however, cannot affect the results of the previous section in this paper, since we have always alternately shielded and irradiated first the anterior and then the posterior sarcoma in the irradiation experiments.

DISCUSSION

Comparison of the nucleic acid metabolism in the irradiated sarcoma with that in a sarcoma protected against the effect of radiation has been found during the first 2 hr after irradiation to be in both cases about one-half of that in the sarcomas of unirradiated rats. The shielding was effectively shown by the control measurements, since the protected sarcoma received a dose of only 13 r at an irradiation level of 2000 r. The inhibition of the nucleic acid metabolism in the shielded sarcoma arises from either circulation of products, formed by the action of X-rays which inhibit the formation of nucleic acid, into the shielded sarcoma or the effect of the radiation on the central regulating mechanism of the rat.

A decrease in the formation of nucleic acid should be accompanied by a lower rate of growth of the sarcoma. The growth of the sarcoma is therefore inhibited, and this also holds good for the shielded sarcoma. From the observation of approximately equal formation of nucleic acid the conclusion can be drawn regarding equality of volume increase. Comparative volume measurements on irradiated and shielded sarcomas in the same rat showed that, during the irradiation period of 4 to 6 days in which a dose of from three to four times 500 r was delivered, the volume increases did not much differ (the shielded sarcomas grow at about 1.3 times the rate for the irradiated ones). In the two periods following, of 8 and 6 days, the irradiated sarcoma showed a substantially lower (one-third) increase than the shielded sarcoma. The formation of nucleic acid in the shielded sarcoma must, therefore, be considerably greater in these periods than that occurring in the irradiated sarcoma. The inhibition of nucleic acid formation which arises in the shielded sarcoma as a result of the action of X-rays is, therefore, much more easily compensated by regeneration processes than that caused by direct irradiation. An indirect effect of X-rays on the growth of the tumours is considerably less permanent than the effect of direct irradiation.

Summary

Two Jensen-sarcomas were injected into each rat. One sarcoma was irradiated with about 2000 r and the other was protected from the effects of radiation by means of lead. Control measurements with a SIEVERT'S ionometer showed that the dose received by the shielded sarcoma amounted at most to 1 per cent of the dose received by the irradiated sarcoma (20 r or less).

The determination of the formation rate of deoxyribonucleic acid in the two sarcomas, using radioactive phosphorus as a tracer, showed that in the first 2 hr after irradiation the formation rate in the shielded sarcoma fell only a little short (20 per cent) of the inhibition of the formation rate in the irradiated sarcoma.

The volume increases of the irradiated and shielded sarcomas were also compared. Three or four times 500 r were supplied in these experiments at 2-day intervals. During the days of irradiation the daily percentage volume increase scarcely differed, but in the subsequent 8 and 6 day periods the value for the irradiated sarcoma was only about one-half that for the shielded sarcoma.

Thus, there is also an indirect effect of X-rays on the sarcoma, but this effect diminishes with time considerably more quickly than the effect obtained as a result of direct irradiation.

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75. ATTEMPTS TO FIND PRODUCTS BLOCKING NUCLEIC ACID FORMATION IN THE CIRCULATION OF AN IRRADIATED ORGANISM

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WE communicated previously⁽¹⁾ the results of some experiments which demonstrate that beside blocking the formation of labelled desoxyribo nucleic acid in irradiated Jensen sarcomata of the rat, a corresponding, though less pronounced, effect is also observed when the sarcomata are entirely shielded from the direct effect of the rays. By placing an ionisation chamber in the cavity of the shielded sarcoma the dose registered amounted to 13 r only, while the second non-shielded sarcoma of the same rat was exposed to a dose of 2000 r. The rate formation of labelled nucleic acid was found to be diminished in both cases.

In the above mentioned experiments the volume increment of both the shielded and the irradiated sarcoma was determined as well. While in the first days after irradiation about the same increment was observed in both sarcomata, in the later phases of the experiment the volume increment of the irradiated sarcoma was found to be only $\frac{1}{3}$ of that shown by the shielded tumor. The effect of indirect irradiation on the Jensen sarcoma is thus in the long run much less pronounced than the effect of direct irradiation.

The indirect action of Roentgen rays is possibly to be explained by a transport of noxious substances formed under the action of radioation from the place of formation by the circulation to the cells on which they act upon. In the present note we describe some experiments which were carried out in the hope to find in the circulation of irradiated animals the presence of irradiation products which by blood transfusion are carried over to a non-irradiated animal and act upon the mechanism of nucleic acid formation in the tissues of the latter.

⁽¹⁾ L. AHLSTRÖM, H. EULER and G. HEVESY, *Ark. Kemi*, **A**, **19** Nr. 13 (1945).

EXPERIMENTAL

In our early experiments, we irradiated rats with Jensen sarcoma with 3000 r, secured about 2 ml plasma or blood and injected the blood immediately into the circulation of a non-irradiated rat. Labelled sodium phosphate was then administered by subcutaneous injection, the rat killed after the lapse of 2 hours and the specific activity of the desoxyribo nucleic acid P and the free P extracted from the sarcome, the liver, the kidneys and the intestinal mucosa was then determined. In view of the experimental difficulties encountered in these experiments we investigated in our later studies the effect of Roentgen radiation on the rate of renewal of desoxyribo nucleic acid in the liver, kidneys and intestinal mucosa of the growing rabbit.

As shown in an earlier investigation⁽¹⁾ the rate of renewal of nucleic acid is diminished under the effect of radiation in the normal organs as well. We carried out experiments with about 4 weeks old rabbits, as in young animals the rate of DNA formation is larger than in the fully grown rabbits.

After the administration of a Roentgendosis, of 7500 to 10,000 r, we have secured 10–25 ml blood from the irradiated rabbit and injected it into the veins of a

TABLE 1. — RATE OF FORMATION OF DESOXYRIBO NUCLEIC ACID IN THE ORGANS OF RABBITS WEIGHING CA 560 GM AFTER TRANSFUSION OF IRRADIATED RESP. NON-IRRADIATED BLOOD OF A SISTER-RABBIT. TIME OF THE EXPERIMENT = 2 HOURS

Volume of transfused blood in ml	Treatment	Specific activity of nucleic acid P in percentage of the specific activity of the free organ-P			Specific activity of the nucleic acid P in percentage of the specific activity of the free plasma-P		
		Liver	Kidney	Intestinal mucosa	Liver	Kidney	Intestinal mucosa
19	Non-irradiated	0.13	0.16	4.2	0.12	0.12	2.1
14	„	1.08	0.23	—	0.86	0.17	4.6
20	„	0.12	0.14	3.1	0.12	0.19	2.7
20	„	0.33	0.11	2.9	0.28	0.17	1.5
11	„	0.54	0.13	2.3	0.39	0.095	—
11	„	0.24	—	2.4	0.18	—	1.8
14	„	0.27	0.41	2.9	0.31	0.54	2.9
19	Irradiated	0.22	0.18	—	0.13	0.16	2.5
13	„	—	0.18	3.3	—	0.31	3.8
11	„	0.13	0.16	2.2	0.28	0.094	2.8
10	„	0.13	0.065	3.0	0.12	0.034	2.8
9	„	1.27	0.087	3.0	—	—	—
23	„	0.14	0.053	3.0	0.082	0.042	1.8
12	„	0.57	0.20	3.0	0.48	0.11	1.6
Non-irradiated							
Mean value		0.39	0.20	3.0	0.32	0.21	2.6
		0.41	0.13	3.0	0.22	0.13	2.5
Irradiated							
Percentage difference between the value obtained after transfusion of non-irradiated and irradiated blood							
		5	35	0	30	39	0

⁽¹⁾ L. AHLSTRÖM, H. EULER and G. HEVESY, *Ark. Kemi A* **19**, Nr. 9 (1944).

non-irradiated sister rabbit from which the same volume of blood was previously removed.

The blood was taken from the carotis, heparin added and the blood injected into the jugularis. To avoid the removal of as much as 20 ml from a rabbit weighing about 560 gm only, we carried out the blood transfusion in steps. We removed for example 6 ml replaced this amount by an equal volume of irradiated blood, waited for 2—3 minutes to obtain a more or less homogeneous mixing of the blood injected with that originally present in the circulation. This procedure was then twice repeated.

Labelled phosphate of an activity of about 20 microCuries was administered by subcutaneous injection immediately after the blood transfusion took place and after the lapse of 2 hours the desoxyribo nucleic acid and the free phosphate of the liver, kidney and intestinal mucosa were extracted. A minor part of the purified and ashed fractions was used to a colorimetric P determination while the bulk of the fractions was applied in activity measurements.

As the rate of renewal of the nucleic acid in the organs of the rabbit is showing appreciable variations, it was necessary to compare the rate of renewal after transfusion of irradiated blood with the rate of renewal observed in a sister rabbit of the same age after transfusion of non-irradiated blood of similar volume. The results obtained are seen in Table 1.

In Table 1 the specific activity of the nucleic acid P is compared both with that of the free organ P and the free plasma P.

The specific activity of the nucleic acid P of the kidney both compared with the specific activity of the free kidney P and the free plasma P is clearly smaller in rabbits which got irradiated blood injected than in the controls. The difference observed is not insignificant in view that in experiments with rats in which the labelled phosphate was administrated two hours after irradiation with about 2000 r, the specific activity of the nucleic acid P extracted from different organs was reduced only to $\frac{1}{2}$ — $\frac{1}{3}$ of the corresponding value found in controls⁽¹⁾.

No definite conclusions can however be drawn from the liver values and clearly no difference is obtained in the case of the intestinal mucosa.

Summary

Blood transfusion was carried out from a growing rabbit irradiated with 10,000 r to a non-irradiated sister rabbit.

Radiophosphorus was administered to the non-irradiated rabbit and after the lapse of 2 hours the specific activity of the desoxyribose nucleic acid phosphorus extracted from the kidneys, liver and intestinal mucosa was compared with the free phosphorus extracted from the corresponding organs and from the plasma.

Control experiments were carried out after blood transfusion from a non-irradiated rabbit to a sister rabbit.

The transfusion of irradiation blood is followed by a slower formation of labelled desoxyribose nucleic acid in the kidneys. No definite result was obtained in the study of the formation of labelled nucleic acid in the liver and a negative result for the nucleic acid extracted from the intestinal mucosa.

⁽¹⁾ L. AHLSTRÖM, H. EULER and G. HEVESY, *Ark. Kemi A* **19**, Nr. 9 (1944).

76. FATE OF THE NUCLEIC ACID INTRODUCED INTO THE CIRCULATION

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WHEN introducing labelled phosphate of negligible weight into the circulation we are labelling the phosphate ions present in the plasma and can follow their path under strictly physiological conditions. Most organic phosphorus compounds introduced into the circulation show a very different behaviour. One of the present writers and ATEN⁽¹⁾ introduced labelled hexosemonophosphate (containing 6 mgm P) into the circulation of the rabbit and found after the lapse of 1 1/2 hours only 0.1 per cent of the hexose-monophosphate (embodying ester) to be present. The hexosemonophosphate was presumably mainly split by contact with the bone and other tissues, a powerful agency producing hydrolysis of hexosemonophosphate being Robinson's bone enzyme⁽²⁾. A substantial splitting of hexosemonophosphate takes even place in the isolated plasma. 23 per cent of the labelled ester are found to be decomposed when hexosemonophosphate containing 1/15 mgm P is shaken with 10 ml of rabbits plasma for 125 min. at 37°. A somewhat larger percentage (31 per cent) is split when the plasma is replaced by blood.

That adenosintriphosphate present in the tissues of the various organs promptly exchanges its labile phosphate groups with non-labelled phosphate present in the tissue, was found in early investigations⁽³⁾ and was recently elucidated more in detail.⁽⁴⁾

In view of the great lability of the terminal and the second phosphorus atoms of the adenosintriphosphate molecule, we can expect injected

⁽¹⁾ G. HEVESY and A. H. W. ATEN, *Kgl. Danske Vidensk. Selsk. Biol. Medd.* **14**, No. 5 (1939).

⁽²⁾ BODANSKY [*J. Biol. Chem.* **118**, 391 (1937)] arrived at the result, that the phosphatase splitting hexosemonophosphate comes from the bone, the kidneys or some other tissues but not from the intestinal mucosa.

⁽³⁾ G. HEVESY and O. REBBE, *Nature* **141**, 1097 (1938); T. KORZYBSKI and J. K. PARNAS, *Soc. Chim. Biol.* **21**, 713 (1939).

⁽⁴⁾ J. SACHS, *Amer. J. Physiol.* **142**, 145, 621 (1944); *Ibid.* **143**, 157 (1945); R. F. FURCHGOTT and F. SHORE, *J. Biol. Chem.* **151**, 65 (1943); E. V. FLOCK and J. L. BOLLMANN, *Ibid.* **152**, 37 (1944); H. K. KALCKAR, J. GEHLINGER and A. MEHLER *Ibid.* **154**, 285 (1944).

adenosintriphosphate containing labelled labile P introduced into the circulation, to behave almost as injected labelled phosphate. MARSHAK and WALKER⁽¹⁾ who investigated the uptake of ^{32}P by the nuclei of the liver tissue, found the same percentage uptake after intravenous injection of labelled sodiumphosphate and that of labelled adenosintriphosphate.

Labelled phosphorylcholine administered by intraperitoneal injection to rats was found to split off at a rapid rate phosphate⁽²⁾. In their study of the metabolism of labelled aminoethylphosphoric acid CHARGAFF and KESTON⁽³⁾ arrive to the conclusion that an enzymic hydrolysis of aminoethylphosphoric acid takes place in the tissues.

That labelled plasma phosphatides introduced into the circulation are not decomposed, resp. are decomposed at a slow rate only, is due to the fact that these are normal components of the plasma, which interchange as such with the tissue phosphatides.⁽⁴⁾

This note contains the results of an investigation of the fate of labelled nucleic acid introduced in the circulation of the rat.

EXPERIMENTAL

To 3 Jensen sarcoma rats together 0.8 milliCurie ^{32}P was administered by subcutaneous injection. After the lapse of 5 days the desoxyribonucleic acid was extracted separately from the pooled livers and the pooled sarcomata. The nucleic acid was purified as described previously⁽⁵⁾. The purification method supplies nucleic acid free from all other phosphorus compounds but its yield is restricted, 18 mgm liver nucleic acid and 196 mgm sarcoma nucleic acid being obtained, having a total activity of 4 microCuries. The average molecular weight of the in the course of the purification process depolymerized nucleic acid was found to be 14,000.

5 mgm of liver nucleic acid in the first, 5 mgm sarcoma nucleic acid in the 11 following experiments, dissolved in 0.5 cc. physiological sodiumchloride solution were injected in the circulation of the rat. After the lapse of 2 hours the rat was killed, the specific activity of the plasma, the total activity of the blood (or plasma), the total and specific activity of the free P, acide soluble P and total P of the liver and the total activity of other organs was determined as well.

(1) A. MARSHAK and A. C. WALKER, *Amer. J. Physiol.* **143**, 235 (1945).

(2) R. F. RILBY, *J. Biol. Chem.* **153**, 535 (1944).

(3) E. CHARGAFF and A. S. KESTON, *J. biol. Chem.* **134**, 515 (1940).

(4) G. HEVESY and L. HAHN, *Kgl. Danske Vidensk.Selsk. Biol. Medd.*, **15**, Nr. 6 (1940); S. L. ZILVERSMIT, G. ENTEMAN and M. C. FISCHLER, *J. Gen. Physiol.* **26**, 333 (1942/43); cf. even F. L. HAVEN and W. F. BALE, *J. Biol. Chem.* **129**, 23 (1939).

(5) H. EULER and G. HEVESY, *Kgl. Dansk. Vidensk. Selsk. Biol. Medd.* **17**, No. 8 (1942).

RESULTS

The weight of the rats and some of their organs is stated in Table 1.

TABLE 1. — FRESH WEIGHT OF RATS AND SOME OF THEIR ORGANS

Weight in gm

Nr. of rat	Rat	Liver	Spleen	Kidneys
I	165	5.14	0.64	
II	195	5.62	0.73	
III	184	5.64	0.99	
IV	185	5.93	0.75	
V	220	6.26	0.88	1.33
VI	192	6.03	0.64	1.09
VII	230	7.60	0.90	1.39
VIII	207	7.69	1.24	1.36
IX	98	2.79	0.42	0.70
X	100	3.25	0.60	0.78
XI	103	2.95	0.40	0.72
XII	100	3.16	0.58	0.73

In Table 2 the phosphorus content of plasma and liver are stated, in Table 3 the percentage ^{32}P administered present in the plasma or blood and some of the organs.

Per gm fresh weight the liver has the highest ^{32}P content, as seen in Table 4.

In Table 5 the ^{32}P content of the total phosphorus and the ^{32}P content of the acid soluble phosphorus of the liver are compared.

TABLE 2. — PHOSPHORUS CONTENT OF PLASMA AND LIVER

Rat	Plasma free P mgm per cent	Liver free P mgm per cent	Liver total P mgm per cent
I	4.7	65	357
II	5.3	61	347
III	5.2	59	416
IV	4.4	57	404
V	4.5	56	376
VI	4.3	46	359
VII	4.1	49	368
VIII	4.2	52	362
IX	}	65	399
X			
XI			
XII		61	393

To each of the outgrown rats I to IV 5 mgm nucleic acid were administered. To the full-grown rats V to VIII and the growing rats IX to XII

TABLE 3. — PERCENTAGE ^{32}P INTRODUCED AS LABELLED DESOXYRIBOSENUCLEIC ACID PRESENT AFTER 2 HR IN PLASMA AND DIFFERENT ORGANS

Rat	Plasma	Liver free P	Liver acid soluble P	Liver total P	Spleen total P	Kidney total P	Plasma (present as free P only)
I	2.2	11.0	14.5	18.0	0.79		0.81
II	8.8	5.6	7.4	9.4	0.48		0.34
III	1.7	8.2	10.6	14.3	0.92		0.57
IV	1.9	8.6	12.1	16.0	0.65		0.58
V	1.1	6.8	9.2	13.8	0.66	2.80	0.56
VI	1.0	3.7	5.5	8.7	0.38	1.46	0.30
VII	0.32	4.3	6.2	9.0	0.44	1.39	0.25
VIII	0.78	6.1	8.7	10.4	0.77	2.04	0.37
IX (blood)	3.5	10.2	13.4	20.7	0.85	4.7	
X (blood)							
XI (blood)							
XII (blood)	2.2	9.5	11.9	18.8	0.75	3.7	

twice that amount. As seen in Table 3 more than $\frac{3}{4}$ respectively $\frac{2}{3}$ of the nucleic acid taken up from the circulation by the liver was split in the course of 2 hours. This is clearly a lower limit. The labelled non-acid soluble fraction of the liver, for example, contains not only the nucleic acid taken up from the plasma but also the labelled phosphatides formed from labelled free phosphate in the liver. At least 4 per cent

TABLE 4. — PERCENTAGE ^{32}P ADMINISTERED AS NUCLEIC ACID PRESENT IN 1 GM FRESH TISSUE

Rat	Liver	Spleen	Kidney
I	3.5	1.2	
II	1.7	0.7	
III	2.5	0.9	
VI	2.7	0.9	
V	2.2	0.8	2.1
VI	1.4	0.6	1.3
VII	1.2	0.5	1.0
VIII	1.4	0.6	1.5
IX	3.4	0.8	3.2
X			
XI	3.1	0.8	2.5
XII			
Average value	2.3	0.78	1.9

TABLE 5. — PERCENTAGE OF THE ^{32}P CONTENT OF THE LIVER PRESENT AS ACID-SOLUBLE ^{32}P

Rat	I	II	III	IV	V	VI	VII	VIII	IX + X	XI + XII
Percentage of ^{32}P content of the liver present in the acid soluble fraction	81	79	74	75	67	63	69	84	65	63

of the total ^{32}P can be estimated to be present as phosphatide ^{32}P in the liver. A non-negligible amount of ^{32}P will be also present as ribonucleic acid formed in the course of the experiment, while the share of ^{32}P incorporated into desoxyribosenucleic acid ^{32}P in the liver of full-grown rats is very restricted. We must furthermore consider that a considerable part of the ^{32}P split off from labelled nucleic acid in the liver is carried into the circulation and from the plasma into the different organs.

The free liver phosphate interact with the free plasma phosphate at a remarkable speed. KALCKAR⁽¹⁾ and his associates record experiments with rabbits taking 5 minutes only, in which after intravenous injection of labelled phosphate the specific activity of the intracellular free liver P varied between 8 and 37 per cent of the specific activity of the free plasma P.

Though the nucleic acid introduced into the circulation was free from other labelled phosphorus compounds, at the end of the experiment the specific activity of the free P of the plasma is showing a considerable

TABLE 6. — SPECIFIC ACTIVITY OF THE FREE PLASMA P IN PERCENTAGE OF THE ACTIVITY OF THE FREE LIVER P AT THE END OF THE EXPERIMENT TAKING 2 HOURS

Rat	I	II	III	IV	V	VI	VII	VIII
Specific Activity of Plasma P in Percentage of the specific Activity of Liver-P	70	44	54	62	64	62	52	63

percentage of the specific activity of the free P of the liver activity as recorded in Table 6.

In our experiments in which labelled sodium phosphate is administered, after the lapse of 2 hours, the specific activity of the free plasma P is somewhat higher than the specific activity of the free liver P. In experiments in which the radiophosphate was administered by subcutaneous injection to rats after the lapse of 2 hours an average

⁽¹⁾ H. M. KALCKAR, J. DEHLINGER and A. MEHLER, *J. Biol. Chem.* **154**, 275 (1944).

ratio of 1.2 was found⁽¹⁾. That in the experiments described in this note, the specific activity of the plasma P amounts on the average to about $\frac{1}{2}$ of the specific activity of the liver P is due to the fact, that in contradistinction to the above mentioned experiments, the labelled free phosphate penetrates from the liver into the plasma.

As soon as the free phosphate reaches the plasma it interchanges with the phosphate of the various organs; with some at a remarkable rate. This interchange leads to very marked lowering of the level of the plasma activity.

From the above considerations it follows that an appreciable part of the free ^{32}P split off from the labelled nucleic acid in the liver is taken up by other organs and the percentage ^{32}P split off from labelled nucleic acid in the liver is even appreciably larger than indicated by the ratio

$$\frac{\text{total acid-soluble } ^{32}\text{P}}{\text{total } ^{32}\text{P}} \text{ present in the liver, recorded in Table 5.}$$

Some phosphate of the labelled nucleic acid introduced into the circulation is also split off in the plasma.

Intrusion of ^{32}P into the Liver Nuclei

Liver nuclei contain several phosphorus compounds, desoxyribonucleic acid, ribonucleic acid, phosphatides and acid-soluble phosphorus compounds⁽²⁾. In the nuclei of regenerating liver tissue of rats weighing 50 gm MARSHAK and WALKER⁽³⁾ found 3 hours after intravenous injection of labelled sodium phosphate an appreciable percentage of the ^{32}P administered (1.54 per cent per gm nuclei) to be present.

When instead of labelled phosphate labelled chromatin was administered a much higher ^{32}P content of the nuclei (5.08 per cent) was found. The result was interpreted as demonstrating the incorporation either of the whole chromatin or parts of it into the nuclei.

While the possibility of some chromatin or some nucleoprotein present in the chromatin entering into the nucleus cannot be excluded, in view of the results obtained in this investigation it is more probable that not labelled nucleoproteids but labelled phosphate split off in the liver and is mainly or exclusively incorporated into the nuclei.

The liver takes up a large part of the plasma-foreign labelled phosphorus compounds introduced into the circulation and splits off phosphate

⁽¹⁾ H. EULER and G. HEVESY, *Sv. Vet. Akad. Arkiv f. Kemi*, A **17**, Nr. 30 (1944).

⁽²⁾ Most of the acid-soluble P of the nuclei is removed during the separation of the nuclei which takes place in an acid solution.

⁽³⁾ A. MARSHAK and A. C. WALKER, *Amer. J. Physiol.* **143**, 235 (1945).

under enzymic action which is partly carried into the circulation and partly enzymically incorporated into the organic P compounds present in the cytoplasm and in the nuclei of the liver. The splitting-off results in a high labelled free phosphate and labile organic phosphate level in the liver tissue. The regenerating liver tissue is very effective in taking up chromatin from the circulation as follows from MARSHAK and WALKER's investigation. They found the liver to take up 26.3 per cent of the ^{32}P administered as chromatin, while only 4.4 per cent of the ^{32}P administered as sodium phosphate was taken up. The main phosphorus compound of the chromatin is desoxyribonucleic acid. The phosphorus of the nucleic acid present in the chromatin taken up by the liver can therefore be expected to show a similar behaviour as the phosphorus of the nucleic acid taken up by the liver, the fate of which is described in this note. In both cases the bulk of the labelled phosphate taken up by the liver is turned into free and acid-soluble organic phosphate, and a part of which is then incorporated into the nuclei.

Splitting-off of the Phosphate Group of Nucleic Acid by the Action of Liver Slices

Liver slices incubated in bicarbonate Ringer solution were found to be very effective in splitting off phosphate from desoxyribonucleic acid.

3.36 gm resp. 3.52 gm liver slices of rats weighing 120 gm were incubated in 9.8 ml phosphate-free bicarbonate ringer solution ($\text{pH} = 7.8$) to which 18.6 mgm labelled desoxyribonucleic acid were added. Oxygen containing 5 per cent CO_2 was led through the Erlenmeyer flask, containing the slices, which was gently shaken for 4 hours at 37° . The slices were then separated by centrifugation.

In the centrifugate 50 mgm non-active desoxyribonucleic acid were dissolved, to facilitate the recovery of the active nucleic acid present in the solution. The nucleic acid was then precipitated by hydrochloric acid dissolved in metanol. After wet ashing and addition of 44 mgm of non-active sodiumphosphate, the phosphorus was precipitated as magnesiumammoniumphosphate.

From the filtrate, after evaporating the metanol and addition of 80 mgm non-active sodiumphosphate, the free phosphate content was recovered as magnesiumammoniumphosphate.

The slices were washed with physiological sodiumchloride solution 4 times, the first washing liquid being added to the centrifugate prior to the precipitation of the nucleic acid. A half of the slices was utilized for the determination of the total ^{32}P content, the other half to the determination of the acid-soluble ^{32}P content. The first named fraction was extracted with 5 ml 10 per cent cold trichloroacetic acid. The ^{32}P content of the different fractions is seen in Table 7.

As seen in Table 7, the non-acid-soluble ^{32}P makes out only 35.0 resp. 33.8 per cent of the total ^{32}P present. At least 65 resp. 66.2 per cent of the nucleic acid added was thus decomposed during the experiment. In fact the actual amount of nucleic acid decomposed is larger, as the active non-acid-soluble fraction contains not only the active desoxy-ribosenucleic acid added but also phosphatides and minute amounts⁽¹⁾

TABLE 7. — ^{32}P CONTENT (IN ARBITRARY UNITS) OF THE DIFFERENT FRACTIONS IN EXPERIMENTS I AND II AFTER INCUBATION OF THE LIVER SLICES IN A BICARBONATE RINGER SOLUTION CONTAINING LABELLED DESOXYRIBONUCLEIC ACID FOR 4 HOURS AT 37°

	I	II
Free ^{32}P in Ringer-solution	3,600	3,120
Acid-soluble ^{32}P in liver slices	738	810
Total acid soluble ^{32}P ..	4,338	3,930
Nucleic acid ^{32}P in Ringer solution	1,950	1,650
Non-acid-soluble ^{32}P in liver slices	377	355
Total non-acid soluble ^{32}P	2,327	2,005
Non-acid soluble ^{32}P of all fractions in percentage of the total ^{32}P content of all fractions	35.0	33.8
Percentage of the total acid-soluble ^{32}P present in Ringer-solution as free ^{32}P	83	79

of active desoxyribo- and possibly larger amounts of ribonucleic acid, formed in the liver slices in the course of the experiment from the active phosphate split off from the active desoxyribosenucleic acid added. Even liver slices are thus very effective in splitting off phosphate from nucleic acid.

It is of interest to remark that the greatest part of the nucleic acid P must have been split off outside the liver cells, presumably on the cell membranes. The greatest part of the free phosphate split off is namely found in the solution. In view of the comparatively high non-active phosphate content of the liver slices the greater part of the free labelled phosphate split off inside the liver cells can be expected to remain inside the cells and not to diffuse into the Ringer solution. In experiments in which labelled free phosphate was added to the Ringer solution⁽¹⁾ 19.5 to 25 per cent of the ^{32}P added was found to have diffused into the liver slices, thus a similar percentage as found in the liver tissue in the above described experiment.

⁽¹⁾ L. AHLSTRÖM, H. EULER and G. HEVESY, *Ark. Kemi, A* **21**, No. 6 (1945).

Summary

Labelled sodiumphosphate is administered to rats inoculated with Jensen's sarcoma. From the sarcoma labelled desoxyribonucleic acid is prepared.

Labelled desoxyribonucleic acid dissolved in physiological sodium chloride solution is injected into the circulation of the rat. After the lapse of 2 hours, an appreciable part of the labelled phosphorus is found to be present in the liver, more than $\frac{3}{4}$ being located in the acid soluble fraction.

The total labelled phosphorus content of the plasma makes out at the end of the experiment only 2 per cent of the ^{32}P content injected as labelled desoxyribonucleic acid; $\frac{1}{4}$ of the plasma ^{32}P is present in the free phosphate fraction.

In experiments in which liver slices were incubated in bicarbonate Ringer solution containing labelled desoxyribonucleic acid the phosphate group of more than $\frac{2}{3}$ of the nucleic acid added was found to be split off in the course of 4 hours; $\frac{4}{5}$ of the total acid soluble ^{32}P being present in the free phosphate fraction of the Ringer solution.

MARSHAK and WALKER found that after the injection of labelled chromatin into the circulation of partially hepatectomized rats, the liver nuclei took up much more ^{32}P than when labelled phosphate was injected. They found also that in the first mentioned case the liver contains a much larger percentage of ^{32}P than in the last mentioned one. The results described in this note suggests the explanation that the accumulation of ^{32}P on the liver nuclei after injection of labelled chromatin, is mostly or wholly due to the intrusion of labelled phosphate split off from the nucleic acid molecules present in the chromatin into the liver nuclei.

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77. FORMATION OF NUCLEIC ACID IN SARCOMA SLICES

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THE numerous types of molecules which occur in the organs have a limited life-time. They decompose and are newly synthesized in the course of the metabolic processes taking place in the organs. Their concentration is the result of synthetic and degradation processes. The application of isotopic tracers makes it possible to break up the resultant into its components. The newly formed molecules are of course labelled when tracers are present. Knowing the total number of molecules present, from chemical analysis, and knowing the number of molecules formed, as shown by the isotopic tracer, the number of molecules degraded and therefore information regarding the two components concerned in the resultant process can be obtained.

In the isolated organs many of the types molecules occurring in them disappear but, of course, at different rates. It has not previously been decided whether the decay of phosphatides, nucleic acid and other molecules in the organs, after these organs have been isolated, is a purely unilateral process or the result of a slow synthetic and a more rapid degradative process. The use of an isotopic tracer, however, permits the detection of a synthetic process, even if it takes place at a very low rate, and thus also the discovery of whether the process is strictly unilateral or not. CHAIKOFF and his collaborators⁽¹⁾ found that radioactive phosphatide molecules are formed in tissue sections of liver, kidneys and intestinal mucosa which have been immersed in a radioactively labelled phosphate solution, and a similar result was obtained by BULLIARD, GRUNDLAND and MOUSSA⁽²⁾ with sections of the suprarenal capsule.

FRIES, SCHACHNER and CHAIKOFF⁽¹⁾ later detected the formation of radioactively labelled phosphatides in Nervus Sciaticus and in cerebral

¹ A. ROBINSON, I. PERLMANN, S. RUBEN and I. L. CHAIKOFF, *Nature* **141**, 119 (1938).

² H. BULLIARD, I. GRUNDLAND and A. MOUSSA, *C. R. Acad. Sci., Paris* **207**, 745 (1938); *Ibid.* **208**, 843 (1939).

sections from rats—which was also observed in one of our early investigations (paper 27)—and, to a small extent, in the cerebral pulp; 300 mgm of nerve substance was immersed in 5 ml of RINGER's solution containing the radioactive phosphate; after 4 hr from 0.44 to 0.72 per cent of the activity was present in the phosphatides isolated from 1 gm of fresh nerve substance. The amount of phosphatide formed during the 4 hr of the test cannot be calculated from these data but the above figures indicate the formation of a not inconsiderable amount of radioactive, and therefore, new phosphatide molecules in the isolated nerve. Control experiments showed that the detected activity was not due to impurities and also provided evidence⁽²⁾ that the formation of radioactively labelled phosphatide molecules in the liver and kidney sections was lowered considerably in the presence of cyanide, azide, hydrogen sulphide or carbon dioxide.

In a later investigation it was also found⁽³⁾ that the formation of phosphatide molecules containing P^{32} is considerably increased (about five-fold) on the addition of glucose, galactose, mannose or fructose to the Ringer's solution containing the radioactive phosphate. It was possible to demonstrate that this increase is to be attributed essentially to an increased phosphatide metabolism and not to accelerated entry of phosphate molecules into the nerve cells. When the tissue was removed from the radioactive Ringer's solution after some time and immersed in a similar inactive solution, there was additional formation of radioactive phosphatide in the nerve tissue only in the presence of glucose and not, on the contrary, in a glucose-free solution. The promoting effect of the glucose on the formation of new phosphatide molecules is presumably connected with the increased oxygen consumption of the cerebral tissue in the presence of glucose. The oxygen consumption of the tissue remains unchanged for a very long time when the solutions contain glucose but decreases rapidly in glucose-free Ringer's solution.

The formation of other compounds, e. g. di-iodotyrosin and thyroxin, in the tissue sections, as well as the formation of phosphatide molecules, was also found by CHAIKOFF and co-workers⁽⁴⁾ to be related to the uninhibited oxygen consumption of the tissue. Inhibition of cytochrome-oxidase by means of cyanide or other impurities has been shown to be harmful to the synthesis of new molecules in tissue sections of the thyroid gland. We shall emphasize at this point that the formation of ^{32}P -containing deoxyribose molecules in sarcoma sections observed by us is disturbed by the presence of cyanide, fluoride or monoiodoacetate.

(1) S. A. FRIES, H. SCHACHNER and J. L. CHAIKOFF, *J. Biol. Chem.* **144**, 59 (1942).

(2) A. TAUROGG, J. L. CHAIKOFF and J. PERLMAN, *J. Biol. Chem.* **145**, 281 (1942).

(3) H. SCHACHNER, V. A. FRIES and J. CHAIKOFF, *J. Biol. Chem.* **146**, 95 (1942).

(4) H. SCHACHNER, A. L. FRANKLIN and J. L. CHAIKOFF, *J. Biol. Chem.* **151**, (1943).

Indications were also obtained that the degradation of cystein in the liver is not a strictly unilateral process.⁽¹⁾ Not only is cystein split into pyruvic acid and ammonia and hydrogen sulphide, but active cystein is formed when active hydrogen sulphide is present. Only about $1/2$ per cent of the cystein molecules present are radioactive.

Finally, mention is made here of the experiments by means of which the formation of glycogen in liver sections was detected⁽²⁾, with pyruvic acid serving as the substrate. Carbon originating from added radioactively labelled sodium bicarbonate was detected in 12 per cent of the glycogen formed. The yield of radioactively labelled glycogen *in vitro* was about the same as that detected in experiments *in vivo*.

DETECTION OF THE FORMATION OF RADIOACTIVE-PHOSPHORUS-CONTAINING DESOXYRIBONUCLEIC ACID IN SARCOMA SLICES

In studying whether nucleic acid molecules are formed *in vitro* we have shaken slices of freshly removed Jensen sarcoma, from rats, with bicarbonate and Ringer's solution, which contained active sodium phosphate, for a period of 4 hr at 37° C. Warburg flasks were used in the first experiments. In each flask were placed 2 gm of slices and 4 cm³ of liquid. The Warburg apparatus was repeatedly filled with oxygen gas (95% O₂ and 5% CO₂). At the end of the experiment the slices were washed repeatedly with an inactive mixture of bicarbonate and Ringer's solution. The slices were homogenized, a small portion was used for determining the free P and its activity, and the remainder was given over to the isolation of nucleic acid. The activity of the Ringer's solution at the end of the experiment was also determined. We have since changed the process, however, by introducing the slices into a 300 cm³ Erlenmeyer flask and by replacing the bicarbonate solution, whose original pH of 7.6 had been disturbed in the course of the experiment, with rat's blood or plasma. After the flasks had been placed in thermostats, the oxygen-carbon dioxide mixture was first of all led through the flasks for 10 min and the active phosphate, with a strength of several microcuries, was then added. Blood has more often been used than plasma in order to obtain a greater volume of liquid and to save the time required in separating the plasma.

⁽¹⁾ C. V. SMYTHEY and T. HALLIDAY, *J. Biol. Chem.* **144**, 237 (1942).

⁽²⁾ J. M. BUCHANAN, A. B. HASTINGS and F. B. NESBETT, *J. Biol. Chem.* **145**, 715 (1942). With regard to the formation of radioactively labelled acid-soluble P compounds in muscle extract refer to J. K. PARNASS, *Bull. Soc. Chim. Biol. Z.* **21**, 1058 (1939); M. O. MEYERHOFF, *Ibid.* **21**, 1094 (1939).

PURIFICATION OF THE NUCLEIC ACID

Since in most cases the activity of the deoxyribonucleic acid P falls far behind the activity of the remaining phosphorus-containing fractions, the nucleic acid should always be carefully purified, even in *in vivo* experiments, from phosphorus-containing impurities. In experiments in which a study is made of nucleic acid formation in tissue slices, there is a still greater demand for purification. Various studies have been designed as checks on the purification.

Active sodium phosphate (50 mgm) (2×10^6 units of activity) was added to the solution containing 236 mgm of crude nucleic acid and the latter was accordingly precipitated. Two further purifications were performed after the addition of 20 and 10 mgm of sodium phosphate, respectively. Further purification was obtained by precipitating twice with methanol containing hydrochloric acid. The pure nucleic acid thus obtained (64.4 mgm) showed an activity of 6.8 relative units (pulses/min in the Geiger tube); 30 mgm of this nucleic acid was reprecipitated yet again with methanol and hydrochloric acid, and the 19.5 mgm finally obtained had an activity of 0.6 units. The above result shows that our purification procedure is effective, since we have found about 100 units in purified nucleic acid samples of similar weight in the experiments showing positive results.

The purification of nucleic acid with respect to free P can accordingly be made quite substantially. Apart from active free P, however, our original fractions also contain active acid-soluble phosphorus compounds, and active phosphatides. We have, therefore, made a study of the completeness with which these impurities may be removed.

The active acid-soluble phosphorus compounds were obtained in the following way: 30 μ c of ^{32}P was injected into a rat. The animal was killed 6 hr later and the acid-soluble P compounds of the Jensen sarcoma were extracted with 10% trichloroacetic acid. One-half of the neutralized active trichloroacetic acid extract was added to the crude nucleic acid which had been extracted⁽¹⁾ from an inactive Jensen sarcoma (9.94 gm). A fraction of the crude nucleic acid was analysed (No. I) and the main part was purified by precipitation with methanol and hydrochloric acid, adding 20 mgm of inactive Na_2HPO_2 to the solution before precipitation. A fraction was again used for analysis (No. II) and the major part of the specimen, dissolved in alkali, was treated with 10 mgm sodium phosphate and precipitated with methanol and hydrochloric acid. Further purifications, the results of which are to be seen in Table 1, were carried out with additions of sodium phosphate.

⁽¹⁾ cf. H. EULER and G. HEVESY, *Kgl. Dansk. Vid. Akad. Biol. Medd.* **17**, No. 8 (1942).

The yield of pure nucleic acid was 31 mgm whereas the weight of the crude amounted to 160.8 mgm (No. I).

The purification of nucleic acid with respect to added active phosphatides was checked in a similar way. An emulsion of radioactively labelled phosphatides was added to the sarcoma extract, and the activity of the

TABLE 1. — PURIFICATION OF CRUDE NUCLEIC ACID WITH RESPECT TO ADDED ACID-SOLUBLE PHOSPHORUS COMPOUNDS

No.	Activity of sample
I	61,700
II	9,250
III	1,675
IV	399
V	41.1
VI	11.9
VII	6.05
VIII	1.92
IX	0.92

nucleic acid obtained after three purifications with methanol and hydrochloric acid was investigated. About 10^5 units of activity were added; the 15 mgm of nucleic acid isolated in this experiment had an activity not exceeding 1 unit. The radioactively labelled phosphatides were prepared from various organs of rats which had been injected 4 hr previously with radioactive phosphate.

DETERMINATION OF THE SPECIFIC ACTIVITY OF THE FREE PHOSPHATE

If the free phosphate takes a direct part in the synthesis of radioactive nucleic acid molecules, the ratio of the specific activity in the nucleic acid P to that in the free P is a measure of the nucleic acid molecules newly formed during the course of the experiment. If a phosphate-containing intermediate product, rather than free phosphate, enters the nucleic acid molecule, it makes no difference to the above inference. The ratio quoted above would not be an exact measure of the newly formed molecules of nucleic acid if the intermediate product were synthesized more slowly than the nucleic acid. In this event, the synthesis of nucleic acid molecules containing no active phosphate would take place for some time in a medium containing active phosphate. There is, however, no evidence in support of this last-mentioned case.

The determination of the amount of nucleic acid formed during the experiment is made more difficult by the impact of quite considerable obstacles in calculating the specific activity of intracellular phosphate for *in vitro* experiments.

The activity of free intracellular phosphorus for *in vivo* experiments may be calculated from the specific activity of the total free P in the tissue and from the specific activity of the free plasma P. We know the magnitude of the extracellular volume and we also know that the phosphate concentration and the phosphate activity of the extracellular fluid correspond approximately to those of the plasma when the time of experiment amounts to a few hours or more. These quantities are determined in every experiment. We also know the free P content of the tissue and its activity. In liver, for instance, when the specific activity of the free tissue P (intracellular plus extracellular) is 1000 and that of the plasma 1500, the liver and plasma contain 20 and 4 mgm of free P, respectively, and when one-quarter of the liver weight consists of extracellular fluid, we obtain a value of 974 for the specific activity of the free intracellular phosphorus. The difference between the specific activities of the intracellular and of the total free P may be considerably greater than in the above case, e. g. in muscle tissue where phosphate slowly penetrates into the cells. In such cases, however, the difference may be calculated likewise by means of a similar procedure.

This procedure cannot be used for *in vitro* experiments. In this instance the tissue is washed with a phosphate-free Ringer's solution in an attempt to remove the extracellular phosphate. In this method it is hardly possible to avoid washing out a part of the intracellular phosphate. This does not nullify the determination of the correct value for the specific activity of the intracellular free phosphate, but decomposition of a part of the existing organic phosphorus compounds possibly takes place during the washing and phosphate is thus split off. This phosphate is probably only slightly active and therefore "dilutes" the activity of the original free phosphate. The result of this circumstance is underestimation of the specific activity of the free intercellular phosphorus and a corresponding overestimate of the calculated amount of nucleic acid formed during experiment.

FURCHGOTT and SHORR⁽¹⁾, when experimenting with sections of heart muscle, found a 32 per cent cleavage of the creatine-phosphoric acid present at the beginning of the experiment in a period of 90 min at 37.55° C, and CHAIKOFF and co-workers⁽²⁾ found a decomposition of

⁽¹⁾ R. F. FURCHGOTT and E. SHORR, *J. Biol. Chem.* **151**, 65 (1943).

⁽²⁾ B. A. FRIES, H. SCHACHNER and J. L. CHAIKOFF, *J. Biol. Chem.* **144**, 59 (1942).

30 per cent of the phosphatides present in liver slices in the course of 6 hr.⁽¹⁾ The value for the specific activity of the free phosphate extracted from tissue sections at the end of the experiment by thorough washing is, for the reasons mentioned above, found to be possibly too low.

FORMATION OF NUCLEIC ACID CONTAINING ^{32}P

We have always performed simultaneously two strictly analogous experiments. One half of the mixture of sarcoma sections was used for one experiment and the remaining half for the second, but in one of these experiments a substance whose inhibiting effect on the formation of nucleic acid could be studied was added to the bicarbonate-Ringer's solution or to the heparinized blood or plasma.

Formation of Nucleic Acid with and without Addition of KCN

In experiment (a) 8.7 gm and in experiment (b) 10.0 gm of tissue sections were placed in 300 cm³ Erlenmeyer flasks containing 30 cm³ of bicarbonate-Ringer's solution (Krebs-Henseleit) at pH 7.4. In experiment (b) 5.7 mgm KCN was also added (pH of the solution also 7.4). After shaking for 15 min at 37.5° C we added to each mixture 0.4 cm³ of physiological saline containing active sodium phosphate and then bubbled a gas mixture of 95% O₂ and 5% CO₂ through the vessels. After 2 hr had passed oxygen and CO₂ were again bubbled through. The time of experiment amounted to 4 hr. At the end of the experiment the sections were washed ten times with inactive bicarbonate-Ringer's solution or physiological saline solution and this was followed by extraction and purification of the nucleic acid:

sample I (without KCN) yielded 9.40 mgm of purified nucleic acid;
sample II (with KCN) yielded 16.5 mgm. The activity of sample I was 84 and of sample II, 0.7.

⁽¹⁾ The facts described make it more difficult to decide the problem of whether or not, for example, the activity of the creatine phosphoric acid P becomes equal to that of the free intracellular P in the course of a relatively short time. Decomposition of a part of the creatine phosphoric acid and other compounds, which are less active than the free intracellular P, lowers the specific activity of the intracellular P and thus narrows the difference between the specific activities of the creatine phosphoric acid P and the true intracellular free P until it more or less vanishes. The results which have been obtained by comparing the specific activities of various other organic P fractions, on the contrary, are fully guaranteed; e. g., we have full information on the relative rates of renewal of the three P atoms in adenosine phosphoric acid (FURCHGOTT and SHORR, *J. Biol. Chem.* **151**, 65 (1943), E. V. BLOCK and J. L. BOLLMAN, *J. Biol. Chem.* **152**, 371 (1944).

Activity per mgm nucleic acid P:

I (without KCN) 128;

II (with KCN) 0.6.

The total activity of the Ringer's solution at the end of experiment

I 0.85×10^6 ;

II 1.05×10^6 .

Activity of 1 mgm of nucleic acid P as a percentage of that contained in the Ringer's solution

I 1.5×10^{-2} ;

II 5.0×10^{-5} .

The above data show that in the absence of KCN an easily detectable portion of the nucleic acid is active and therefore newly formed, and that in the presence of KCN the formation of new molecules of nucleic acid is decreased to $1/260$ of that observed in the absence of this addition. In another experiment 1.3×10^{-2} of the final activity of the Ringer's solution was found per mgm of nucleic acid P.

In later experiments the Ringer's solution has been replaced by heparinized rat's blood or plasma, and the activity of 1 mgm of free P in the sections has also been determined at the end of the experiment in order to permit calculation of the percentage of newly formed nucleic acid (cf. the discussions on p. 774).

Formation of Nucleic Acid in the Presence and Absence of NaF

These experiments also were performed at 37°C ; 7.56 gm of sarcoma sections were immersed in 15 cm^3 of rat's blood in both experiments, (a) and (b), 18.9 of radioactively labelled sodium phosphate at pH 7.4 and 8.1 mgm of NaCl at the physiological concentration being added in experiment (a). In experiment (b) the same amount of sodium phosphate was used but 6 mgm of NaF was added instead of NaCl. A mixture of oxygen and CO_2 was passed through the Erlenmeyer flasks for 15 min before addition of the activity and then for 5 min in each subsequent hour, at 37°C . After breaking off the 4 hr experiment the activity of the purified nucleic acid, its P content, the activity of the free P of an average sample of the slices and the P content of the latter were determined. The activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of free sarcoma P was found to be:

(a) without NaF, 0.126;

(b) with NaF, 0.026.

Activity of the free P in the whole of the tissue as a percentage of the activity in the whole of the plasma plus the whole of the free P in the tissue:

(a), 23.0;

(b), 33.5.

and calculated per gm of tissue:

(a), 4.6;

(b), 4.4.

Approximately 0.1 per cent of the nucleic acid present was therefore synthesized during the experiment, and the addition of NaF reduced the amount synthesized to about one-fifth of this value. In 1 gm of tissue there was more than 4 per cent of the activity present at the end of the experiment in the plasma plus sarcoma section system.

A significant amount of activity also penetrated into the blood corpuscles, but this is not of interest in connexion with the above discussion.

Formation of Nucleic Acid in the Presence and Absence of Monoiodo-Acetic Acid

In experiments (a) and (b), 4.31 and 3.72 gm of tissue, respectively, were immersed in 16.2 cm³ of blood; 5 cm³ of physiological saline solution containing ³²P was added to the blood in experiment (a), while in experiment (b) 2 cm³ of physiological saline solution and 3 cm³ of an aqueous solution, which contained 10 mgm of sodium iodoacetate adjusted to pH 7.4 by addition of NaOH, were added to the blood. The activity of 1 mgm of nucleic acid P as a percentage of the activity of 1 mgm of free sarcoma P was found to be:

(a) 0.0338;

(b) 0.00076.

Formation of Nucleic Acid with and without Addition of Oestrone

In experiments (a) and (b), respectively, 3.3 and 3.9 gm of tissue section were immersed in 16.5 cm³ of blood to which active sodium phosphate (2 mgm P in physiological saline) had been added; 2 mgm of oestrone dissolved in 0.1 cm³ of alcohol was added to the experimental solution (b); 0.1 cm³ of alcohol, without oestrone, was added to solution (a). The activity of 1 mgm of nucleic acid P, expressed as a percentage of the activity of 1 mgm of free sarcoma P, was found to be:

(a) without oestrone, 0.097;

(b) with oestrone, 0.074.

The percentage of the activity of the whole tissue present in the plasma plus sarcoma tissue at the end of the experiment was:

(a) 22.3;

(b) 20.8;

and per gm tissue

(a) 6.76;

(b) 5.42.

The presence of oestrone cannot, therefore, substantially affect the synthesis of nucleic acid molecules.

Formation of Nucleic Acid in the Presence and Absence of Caffeine

In this experiment 5.52 gm (arrangement (a)) and 5.53 gm (arrangement (b)) of tissue sections were immersed in 19.5 cm³ of blood which contained 1 mgm of active sodium phosphate; 0.66 cm³ of physiological saline solution containing 20 mgm of caffeine was added to (b), and the same volume of saline solution without caffeine to (a). Time of experiment, 4 hr, temperature 37° C. The results found were:

Activity of 1 mgm nucleic acid P as a percentage of the activity of 1 mgm of free sarcoma P:

(a) 0.104;

(b) 0.147.

Percentage of the activity of the plasma plus the free P activity of the tissue sections present at the end of the experiment as free P in the tissue:

(a) 21.6;

(b) 22.2;

and per gm of tissue

(a) 3.91;

(b) 4.02.

Caffeine does not, therefore, significantly affect the formation of radioactive molecules of nucleic acid.

Formation of Nucleic Acid in the Presence and Absence of Colchicine

Tissue sections (6.5 and 6.0 gm in experiments (a) and (b), respectively) were immersed in 18.5 cm³ of blood to which had been added 1 mgm of radioactive sodium phosphate; 4 mgm of colchicine was also added to solution (b). Time of experiment, 4 hr, temperature, 37° C. The percentage ratio for 1 mgm of nucleic acid P to that of 1 mgm of free tissue P was:

(a) without colchicine 0.175;

(b) with colchicine 0.172.

The total sarcoma activity as a percentage of the plasma activity plus the sarcoma activity was:

(a) 25.4;

(b) 25.8.

and per gm sarcoma tissue

(a) 4.91;

(b) 4.30.

The addition of colchicine therefore does not affect the formation of nucleic acid. A negative result was also obtained on adding diethylstilboestrol (addition of 3 mgm of a sample prepared by Dr. E. ADLER).

Formation of Nucleic Acid without Additives

In one experiment in which 5.38 gm of sarcoma sections was shaken with 25 cm³ of blood the percentage ratio of the activity of 1 mgm of nucleic acid P to that of 1 mgm of free P was 0.033.

Metabolism of Nucleic Acid in Liver Sections

The determination of the metabolism of nucleic acid in liver slices lies outside the scope of this investigation. Yet a determination of this kind has been performed as a sort of purity check. The amount of labelled nucleic acid formed in the liver in a period of 2 hr amounts to only about 0.1 per cent of the total amount of nucleic acid present. A considerably smaller value must be expected in liver slices. A weight of 10.6 gm of liver slices was shaken for 4 hr with 24 cm³ of plasma, which contained 15 μ c of ³²P, at 37° C and the percentage ratio of the specific activity of the nucleic acid P and of the free tissue P was then determined. This was found to be 0.0085. The percentage ratio of the nucleic acid P and of the plasma P was 0.0021. These figures show that the formation of new molecules of nucleic acid in the liver sections is lower by a factor of 10, or more, than in the liver. These data provide further evidence that the purification of our nucleic acid from phosphorus-containing impurities is efficient.

AMOUNT OF NUCLEIC ACID DECOMPOSED PER HOUR IN SARCOMA SECTIONS

In order to obtain information on the rate at which nucleic acid decomposes in sarcoma sections, a determination of the nucleic acid content has been made immediately after killing a rat. Some toluene was added to another part of the sarcoma and, after keeping the tissue for 24 hr

at 20°, the determination of nucleic acid was performed similarly. The method of "isotopic dilution" was applied to this determination.

After 40 min boiling of the purified tissue (5 gm) with 5% sodium hydroxide solution, 10 mgm of radioactive nucleic acid was added to the sample and the nucleic acid was isolated after repeated purification. The nucleic acid content of the tissue sample can be calculated from the specific activities of the added and isolated nucleic acid and from the amount of nucleic acid added.

The added amount of nucleic acid had an activity of 461 pulses/min per mgm P, while the corresponding value for the isolated nucleic acid amounted to 28.2; 9.9 mgm of active nucleic acid had been added. The required content of nucleic acid in the purified sarcoma tissue (5 gm) therefore amounts to:

$$x = 9.9 (461/28.2 - 1) = 152$$

or 30.4 mgm of nucleic acid per gm of sarcoma tissue.

The analysis of the sample which had been kept for 24 hr yielded:

$$x = 10.0(1641/36.0 - 1) = 118$$

or 23.6 mgm of nucleic acid per gm of sarcoma tissue.

Only about one-quarter of the nucleic acid content was therefore decomposed during the 24 hr storage.

The data themselves (but not, on the contrary, the ratio) need to be treated with great caution. The use of the isotope dilution method assumes, of course, that the added radioactive sample behaves in all respects like the nucleic acid originally existing in the prepared substance. Now the degree of polymerization of the multiply purified (a treatment which cannot be relinquished) nucleic acid may differ considerably from the degree of polymerization of the nucleic acid present in the crude alkaline extract, and it is conceivable that in the purification of the preparation a greater or smaller fraction of the added radioactive nucleic acid than of that originally present enters into the individual precipitations.

Growing sarcomas weighing 10 to 17 gm from rats weighing from 106 to 113 gm were used in the experiments which have been described. The radioactive nucleic acid was obtained from the sarcomas of such animals which had been injected 2 days previously with radioactive sodium phosphate.

DISCUSSION

The following values have been obtained for the percentage ratio of 1 mgm nucleic acid P to 1 mgm free sarcoma P:

0.126
0.024
0.097
0.104
0.175
0.033

A quantitative comparison of these data with the values from *in vivo* experiments is made more difficult by, among other things, the time of experiment for sarcoma sections being 4 hr whereas the radioactive nucleic acid is probably formed chiefly during the first hour. Yet the conclusion can be drawn from the results that the amount of nucleic acid built up in the sarcoma sections is about one-twentieth of that synthesized *in vivo* in the sarcoma. The incorporation is markedly inhibited by poisons such as cyanide, fluoride or monoiodoacetic acid, whereas colchicine, oestrogenic substances and caffeine do not exercise any appreciable effect.

Summary

If sarcoma sections are shaken for 4 hr at 37° C with blood, plasma or bicarbonate-Ringer's solution which contains radioactive sodium phosphate, the deoxyribonucleic acid extracted from the sections and then purified exhibits radioactivity. The ratio of the specific activity of the desoxyribonucleic acid P to that of the free P isolated from the sarcoma tissue has been found to be on the average 1000. The metabolism of desoxyribonucleic acid in sarcoma sections is therefore about one-twentieth of that found in a living animal in a 2 hr experiment.

Cyanide, fluoride and monoiodoacetic acid inhibit the formation of radioactive desoxyribonucleic acid, but colchicine, oestrone, diethylstilboestrol and caffeine do not.

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78. APPLICATION OF LABELLED SUBSTRATES IN THE STUDY OF ENZYMIC PROCESSES

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THE application of labelled substrates in the study of enzymic processes has two advantages:

(a) The sensitivity of the determination of the enzyme can be much increased.

(b) The determination of the reaction product, for example, radio-phosphate in phosphatase studies, is not influenced by non-labelled phosphate, even if present in a high concentration. Accordingly, the determination is based on the appearance of radioactivity in an initially inactive system, and not on the increase in concentration of the inorganic phosphate. In our experiments we used labelled desoxyribonucleic acid and studied the respective actions of blood plasma phosphatase, sarcoma phosphatase, and intestinal mucosa phosphatase on the labelled substrate.

PREPARATION OF SUBSTRATE

Labelled sodium phosphate of an aggregate activity of 10 millicuries was administered to rats which were sacrificed after a few days. Then the desoxyribonucleic acid was extracted from the Jensen-sarcoma, the liver, the intestinal mucosa, or other organs, and was carefully purified so as to reprove all other labelled phosphorus compounds, as described previously⁽¹⁾. The best yields in respect of both quantity and activity are obtained when the nucleic acid is extracted from the sarcoma or the intestinal mucosa. No advantage is gained by letting the rat live longer than 3 to 4 days, as we have to reckon not only with a decay of the activity of radiophosphorus at a rate of about 4 per cent per day, but also with an exodus of the labelled phosphate from the nucleic acids and other compounds of the organs. This exodus is particularly pronounced in such tissues as the intestinal mucosa in which the nucleic acid content is renewed at a comparatively high rate.

⁽¹⁾ H. EULER and G. HEVESY, *Kgl. Danske Vid. Selsk. Biol. Medd.* **13**, No 8 (1945).

1 mgm of the desoxyribonucleic acid obtained had an activity of up to 2000 counts per minute. Thus, 0.05 mgm of P split off from this substrate was determined without difficulty by activity measurements. The nucleic acid, which is highly polymerised in the beginning, becomes depolymerised to a certain extent in the course of the purification process, and had a molecular weight of about 14,000 only.

In our investigations we use a few mgm or less of labelled substrate, which was added to the plasma of a rat, and the plasma was kept at 37° for a definite time. After cooling with ice, trichloroacetic acid was added, and both the precipitate and the filtrate were secured. The precipitate contained the non-split nucleic acid, while the filtrate was found to contain not only labelled inorganic phosphate but also an organic acid-soluble fraction containing labelled phosphate. By comparing the activities of the different fractions we obtained figures for the percentage formation of inorganic and organic acid-soluble P, due to enzymic action.

The inorganic phosphate was precipitated from an aliquot of the filtrate after adding 80 mgm of inactive sodium phosphate as ammonium magnesium salt. Another aliquot was used for colorimetric determination of inorganic phosphorus. A third aliquot was ashed, and from the solution obtained the P was precipitated as described above. The difference between the total acid-soluble P and the inorganic P corresponds to the organic acid-soluble P split off owing to the action of enzymes from the nucleic acid added.

EFFECT OF BLOOD PLASMA PHOSPHATASE ON NUCLEIC ACID

To begin with, we compared the phosphatase activities of the blood serum of 6 weeks old normal rats and rats with Jensen-sarcomata. 7.02 mgm of labelled desoxyribonucleic acid was dissolved in 15 ml of serum. The radioactivity of 2 ml was measured at once, and the radioactivity of an equal volume of solution was determined after it had been kept at 37° for 2 and 24 hours respectively. The nucleic acid concentration was 1.17 mgm (0.1 mgm nucleic acid P) per ml of plasma. The protein and unchanged nucleic acid present were precipitated by adding 2 ml of 10 per cent trichloroacetic acid, the precipitate was centrifuged, washed with further 2 ml trichloroacetic acid and the activity of the sample determined. An aliquote of the filtrate was ashed and its total phosphorus content secured as magnesiumammonium phosphate. From an other aliquote of the filtrate the inorganic phosphate was precipitated. We investigated thus 3 fractions:

- (a) Protein, (b) Inorganic P, (c) Total acid-soluble P.

The results are seen in Table 1. In the last column of this table the difference between the total acid-soluble P and the inorganic P is stated.

From the figures of Table 1 follows, that a minor amount of the nucleic acid is not precipitated by addition of trichloroacetic acid alone

TABLE 1. — EFFECT OF PLASMA PHOSPHATASE ON DESOXYRIBONUCLEIC ACID

Time of experiment, hours	Percentage ^{32}P present in		
	Protein	Inorganic P	Organic acid-soluble P
0	92	5.2	2.0
0	94	5.8	0.6
2	73.8	21.2	5.2
2	74.4	20.2	5.7
24	13.7	84.2	2.0
24	13.6	86.6	0.6

(it is stated that better results are obtained by adding besides trichloroacetic acid, hydrochloric acid as well)⁽¹⁾. After the lapse of 2 hours, about $\frac{1}{6}$ of the phosphate was split off under the action of the plasma phosphatase, and after the lapse of 24 hours, most of the phosphate of the nucleic acid was present as inorganic phosphate. Small amounts of organic acid-soluble P were present as well. As described on p. 786 this fraction is much increased when plasma phosphatase is replaced by intestinal mucosa phosphatase.

TABLE 2. — PERCENTAGE PHOSPHATE SPLIT OFF FROM NUCLEIC ACID ADDED TO PLASMA OF NORMAL AND OF JENSEN-SARCOMA RATS. T = 37° C

Time in hours	Plasma	Percentage Phosphate Split off
2	normal	21; 20.1
2	sarcoma	22.5; 21.9
3½	normal	24.1; 24.0
3½	sarcoma	18.6; 11.6
4	normal	47.0; 39.7; 32.2; 34.8
4	sarcoma	35.0; 22.6; 32.4; 33.3
8	normal	51.5; 53.5
8	sarcoma	49.3
22	normal	76.3; 79.0
22	sarcoma	68.8; 66.1

⁽¹⁾ G. SCHMIDT and S. TANNHAUSER, *J. Biol. Chem.* **161**, 83 (1945).

A comparison of the phosphatase activity of the serum of normal and of sarcoma rats leads as recorded in Table 2 to the result that in several cases the activity of sarcoma rat plasma is lower than that of controls but that the difference is not pronounced, the mean values for the percentage phosphate split off being 41.9 and 34.7 respectively.

EFFECT OF ADDITION OF MAGNESIUM ON THE PHOSPHATASE ACTIVITY

In the first set of experiments (I) 0.192 mgm nucleic acid were dissolved in 4 ml serum, in the second set (II) 1 mgm in 1 ml plasma. MgSO_4 was then added amounting to 0.001 mol. per liter. As it was to expect the addition of magnesium salt leads to an increased splitting off of inorganic phosphate. However, as seen in Table 3 the effect observed is not very pronounced and is larger in the experiments in which more substrate was used.

TABLE 3. — EFFECT OF MAGNESIUM ON THE PHOSPHATASE ACTIVITY

Experiment	Time in hours	Plasma	Percentage inorganic P split off		Percentage organic acid-soluble P produced	
I	4	control	30.8;	35.8	4.0;	16.2
	4	+ Mg	34.9;	37.2	21.1;	15.0
II	4	control	21.8;	19.5	15.3;	15.2
	4	+ Mg	36.8;	37.6	23.9;	23.8

Addition of 0.012 ml of 0.1 molar NaF to each ml serum was without effect on the formation of inorganic phosphate or of organic acid-soluble phosphorus compounds. The centrifugate contained 53.5 and 53.5 per cent of the total ^{32}P added as nucleic acid as inorganic P after the lapse of 8 hours without addition of fluoride, while 53.6 and 55.0 per cent were found when fluoride was added. The figures for the total acid-soluble P were 60.9; 60.7 and 61.9; 61.0 resp.

Nor had addition of 0.1 ml 0.02 molar Na_2HAsO_4 to each ml serum containing 0.16 mgm nucleic acid any pronounced effect.

EFFECT OF INTESTINAL MUCOSA EXTRACT ON NUCLEIC ACID

The intestinal mucosa extract used in our experiments was prepared from rats' intestine. The mucosa tissue was suspended in 5 parts of 87 per cent glycerin solution and shaken for an hour. One hundredth volume of 10 per cent NaOH was then added and the extract kept at room tem-

perature for 2 days. The liquid obtained after filtration through cloth and addition of toluene was stored in an ice-box.

The non-split nucleic acid present in the solution after treatment with intestinal enzyme is precipitated with cold trichloroacetic acid. The precipitation is much facilitated by simultaneous precipitation of proteins. We found it very convenient to add blood plasma to the enzyme extract containing the radioactive substrate, previous addition of trichloroacetic acid. As we base all our determinations on radioactive measurements, the presence of phosphorus compounds in the plasma does not interfere with our determinations of the inorganic P and total organic acid-soluble P split off from the labelled nucleic acid added.

As in the experiments with blood plasma, described above, the intestinal mucosa extracts not only split off inorganic P from nucleic acid but also organic acid-soluble phosphorus compounds, which are presumably a mixture of nucleotids of a low degree of polymerization. The appearance of organic acid-soluble phosphorus compounds is more

TABLE 4. — EFFECT OF INTESTINAL MUCOSA
EXTRACT ON LABELLED NUCLEIC ACID

Temperature 37°C. Amount of substrate in
sample = 0.70; 3.6; 1.2; 1.1 mgm

Time in hours	Percentage ^{32}P found as	
	inorganic P	organic acid-soluble P
(a) Extract 3 days old		
1	1.5	9.4
1	1.6	8.8
4	14.8	22.6
4	14.2	22.6
7	31.8	25.9
7	30.6	29.6
(b) Extract 10 days old		
2	17.3	10.9
2	20.9	3.7
4	24.7	8.9
4	25.5	9.5
(c) Extract 10 weeks old		
1	2.7	1.0
4	15.8	1.4
6	23.1	0
(d) Extract 10 weeks old		
1	2.4	4.6
4	11.0	1.0
6	18.5	0

pronounced when using freshly prepared extracts, than when old extracts are applied (cf. Table 4). This observation suggests that the enzyme splitting off inorganic P from nucleic acid is not identical with the enzyme which is responsible for the production of organic acid-soluble compounds. The presence of 2 distinct enzymes acting on polynucleotids was already observed by LEHMANN—ECHTERNACHT⁽¹⁾.

The figures stated in Table 4 are thus corrected for the zero-value.

While the mean value obtained for the ratio

$$\frac{\text{organic acid-soluble } P}{\text{inorganic } P} = 1.5$$

when applying 3 days old enzyme, this ratio declines to 0.37 when the enzyme is 10 days old, and to 0.11 using 10 weeks old enzyme extracts.

EFFECTS OF SARCOMA EXTRACTS ON LABELLED NUCLEIC ACID

We compared the effect of enzymes extracted from the fresh tissue and necrotic tissue of growing benzpyrene sarcoma and of retrograde Jensen-sarcoma. As seen in Table 5, no difference of any significance is shown by the 3 extracts.

TABLE 5. — EFFECT OF SARCOMA EXTRACTS ON LABELLED NUCLEIC ACID

Time = 2½ hours. T = 37° C. Substrate = 1 mgm nucleic acid (in 2 ml)

Sarcoma	Percentage ³² P present as	
	inorganic P	organic acid-soluble P
Growing fresh	8.2	17
Growing necrotic	9.5	17
Regressive	8.3	15

The application of labelled substrates has especially great advantages when we are faced with the problem to determine the enzymic activity of very weak preparations as we can restrict ourselves to the application of minute amounts of substrate.

In our experiments having only radiophosphorus samples of small activity at our disposal, we had to apply P containing labelled substrates

⁽¹⁾ H. LEHMANN-ECHTERNACHT, *Z. Physiol.Chem.* **269**, 169, 187 and 201 (1941); Cf. also EULER, HAHN and SALUSTE, *Sv. Vet. Akad. Arkiv f. Kemi A* **24**, No. 5 (1946).

containing 0.05 mgm P and in some cases even more. But even these amounts compared favourably with the amount of substrates (1—2 mgm P), applied in experiments using the usual chemical methods.

In the possession of highly active radiophosphorus preparations much more active substrates could be used and their weight correspondingly reduced.

Summary

The authors demonstrate that labelled substrates can be applied with advantage in enzymic studies. This is shown by investigating the effects of blood plasma, intestinal mucosa and sarcoma extracts on labelled desoxyribonucleic acid.

The ratio of the organic acid-soluble P to inorganic P obtained from preparations subjected to the action of an intestinal mucosa extract, was found to increase with the age of the preparation.

The enzyme responsible for the splitting-off of organic acid-soluble molecules from desoxyribonucleic acid was found to be less stable than the enzyme responsible for the splitting-off of inorganic phosphate.

COMMENT ON PAPERS 73 TO 78

IN most of our experiments we exposed rats to irradiation and injected them after exposure with ^{32}P . They were then killed at intervals of from 30 min to several days. In some of our experiments we irradiated injected animals that were tied to a table throughout the experiment. Formation of labelled DNA was found (AHLSTRÖM *et al.*, 1944) to be much more strongly depressed in these experiments than in the usual ones. It was, however, soon found (paper 73) that this interference with DNA formation was not due to exposing the rats to uninterrupted irradiation, but to circulation disturbances which prevented the radioactive indicator from reaching the sarcoma.

When the right flank of a rat having tumours both in the right and left flank was irradiated, ^{32}P incorporation into the DNA of the left flank was found to be diminished as well (paper 74). This result induced experiments in which blood of heavily exposed rabbits was transfused to sister rabbits (paper 75). The presence of DNA formation blocking components in the circulation could not be determined with certainty (paper 75).

From Jensen-sarcoma prepared DNA was injected into the circulation of the rat. After the lapse of 2 hr the total ^{32}P content of the plasma was reduced to 2 per cent of the amount of ^{32}P injected (paper 76). MARSHAK and WALKER (1945) observed that after the injection of ^{32}P labelled chromatin into the circulation of partially hepatectomized rats, the liver nuclei took up much more ^{32}P than when labelled phosphate was injected. We suggested the explanation that the accumulation of ^{32}P in the liver nuclei after injection of labelled chromatin is mostly or wholly due to the splitting off of phosphate from the chromatin in the liver, the phosphate being then incorporated into the liver nuclei. Our explanation proved to be erroneous. Labelled DNA found application in the study of splitting off phosphorus from the latter under the action of enzymes present in tissue extracts (paper 78).

The incorporation of ^{32}P into DNA of surviving sarcoma slices was also studied (paper 77). In experiments taking 2 hr incorporation was found to make out one-twentieth of the value observed in *in vivo* experiments. Addition of oestron, diethylstilboestrol or caffenin to the incubating fluid does not influence the rate of labelled DNA formation, addition of cyanide, fluoride or moniodoacetic acid suppresses it.

References

- A. MARSHAK and A. C. WALKER (1945) *Amer. J. Physiol.* **143**, 235.
L. AHLSTRÖM, H. EULER and G. HEVESY (1944) *Ark. Kemi* **18 B**, 13 No. 13.

Originally published in *Nature*, **163**, 869 (1949).

79. EFFECT OF X-RAYS ON THE INCORPORATION OF CARBON-14 INTO DESOXYRIBONUCLEIC ACID

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It was observed by several experimenters⁽¹⁾ that the incorporation of phosphorus-32 into desoxyribonucleic acid of the Jensen-sarcoma, and also of normal organs, of the rat is markedly reduced by irradiation with an X-ray dose of some hundred Roentgen. This result strongly suggests the explanation that the rate of formation of desoxyribonucleic acid is reduced by the effect of ionizing radiation. We studied recently the effect of X-rays on the incorporation of carbon-14 into the purines of desoxyribonucleic acid in the organs of rapidly growing rats.

About five microcuries of carbon-14 incorporated with the carboxyl group of sodium acetate were injected subcutaneously to each of 91 one- to three-week-old rats. The rats were killed after the lapse of six hours, and the desoxyribonucleotides were separated from the proteins by extraction with hot trichloroacetic acid⁽²⁾; the purines obtained by hydrolysis were twice precipitated as silver salts and their radioactivity was measured. Previous to the extraction of the kidney and muscle proteins, 20 mgm of desoxyribonucleic acid, kindly presented by Prof. HAMMARSTEN, were added to the trichloroacetic acid extract. Half the rats were previously irradiated with a dose of 950 r., the voltage applied being 165 kV.

As shown by the figures in the accompanying table, the amount of labelled purines formed in the irradiated rats was about half the amount formed in the controls, which is very similar to the reduction in the incorporation of phosphorus-32 observed earlier⁽¹⁾.

The rate of incorporation of carbon-14 into the proteins was not reduced by irradiation; even some increase was observed, which may be due to a decrease in the metabolic-rate produced by irradiation and leading to an increased level of carbon-14 in the liver and intestinal mucosa, corresponding to a greater incorporation of carbon-14 into the proteins of these organs. The fact that, despite the increased carbon-14 content of the proteins of the irradiated rat, we observed a markedly reduced incorporation of carbon-14 into the purines of desoxyribonucleic acid of such rats clearly indicates a reduced rate of formation of the

RATIO OF CARBON-14 CONTENT OF 1 MGM OF PURINE ISOLATED FROM DESOXYRIBO-
NUCLEIC ACID OF NON-IRRADIATED AND IRRADIATED RATS SIX HOURS AFTER AD-
MINISTRATION OF ABOUT 0.4 MGM OF LABELLED CARBON AS ACETATE TO EACH OF
91 ONE- TO THREE-WEEK-OLD RATS

O r g a n	Ratio of ^{14}C content of purine from desoxyribonucleic acid iso- lated from non-irradiated and irradiated rats, respectively				Ratio of ^{14}C content of 1 mgm protein from organs of non-irradiated rats, respectively		
	Group				Group		
	1	2	3	4	2	3	4
Intestinal mucosa	2.3	2.0	2.2	1.5	0.78	0.80	0.90
Liver	2.3	2.0	1.5	1.5		0.83	0.50
Muscles			3.1	1.8		0.91	1.0
Kidneys	1.0		3.0	3.7		1.0	1.0

Percentage of administered ^{14}C incorporated in 1 mgm intestinal mucosa:

	Purine		Protein	
	min.	max.	min.	max.
Non-irradiated	3.7×10^{-5}	2.4×10^{-4}	4.1×10^{-4}	6.3×10^{-4}
Irradiated	1.7×10^{-5}	1.3×10^{-4}	5.2×10^{-4}	7.8×10^{-4}

last-mentioned compound in the irradiated animal. The observation that the rate of formation of a main constituent of cell nuclei is reduced appreciably after irradiation, helps to explain the blocking effect of irradiation on cell division and the formation of anomalous mitotic products in the irradiated tissue.

References

1. A survey of earlier work has been given by G. HEVESY, *Rev. Mod. Phys.* **17**, 102 (1945). See also H. B. JONES, personal communication (1946), J. S. MITCHELL and B. E. HOLMES, *Ann. Rep. British Empire Cancer Campaign* **9**, 136 (1947); B. E. HOLMES, *Brit. J. Radiol.* **20**, 450 (1947).
2. W. C. SCHNEIDER, *J. Biol. Chem.* **161**, 293 (1945).

Originally communicated in *Nature*, **164**, 269 (1949).

80. EFFECT OF X-RAYS ON THE INCORPORATION OF CARBON-14 INTO ANIMAL TISSUE

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IN a recent note⁽¹⁾, it was shown that irradiation of rats with X-rays diminishes very pronouncedly the rate of incorporation of carbon-14 into desoxyribonucleic acid. Simultaneously, an *enhanced* incorporation of carbon-14 into the total proteins of the organs was observed. This observation induced the investigation of the effect of irradiation on the incorporation of carbon-14 in the whole tissue.

About one microcurie of carbon-14 incorporated with the carboxyl group of sodium acetate was injected subcutaneously into each of an aggregate number of 180 one- to three-week-old rats and full-grown mice, respectively. The animals were killed after the lapse of about eight hours, and the radioactivity of the dried kidney, intestinal mucosa, liver and muscle tissues was determined. Half the animals had been previously irradiated for three minutes with a total dose of 950 r. (in air), the voltage applied being 165 kV. A significant increase in the incorporation of carbon-14 in every sample investigated was observed. The figures in the accompanying tables show some of the results obtained in experiments with rats (*A*) and mice (*B*).

The above results indicate that, while the blocking of the formation of nucleoproteins is probably the most conspicuous biochemical effect of X-rays, the total metabolic pattern is influenced as well.

A possible explanation of at least part of the enhanced incorporation of carbon-14 into the tissue of irradiated animals is that in such animals the basal metabolism is slightly decreased. Such a process would lead to a higher activity-level in the organism which, in turn, would cause an increased incorporation of carbon-14 into organic processes.

Gould *et al.*⁽²⁾ collected labelled carbon dioxide exhaled by full-grown rats following the administration of labelled acetate. During the first hour, they found a 50 per cent reduction in the specific activity of carbon dioxide every 15 minutes, and interpreted this observation as being primarily a dilution of isotopic carbon dioxide of the body fluids with non-isotopic carbon dioxide of metabolic processes. In the course of

TABLE A. — INCORPORATION OF CARBON-14 IN THE TISSUES OF RAPIDLY GROWING RATS FOLLOWING THE ADMINISTRATION OF LABELLED ACETATE (DOSE 950 R.)

O r g a n	Arbitrary activity figures	Per cent increase in carbon-14 incorporation due to irradiation
Kidneys Control	22.05	16.8
Irradiated	25.74	
Intestinal mucosa Control	12.83	31.8
Irradiated	16.93	
Liver Control	11.20	13.5
Irradiated	12.70	
Muscles Control	22.05	16.7
Irradiated	25.74	

TABLE B. — INCORPORATION OF CARBON-14 WITH THE TISSUES OF FULL-GROWN MICE

O r g a n	Arbitrary activity figures	Per cent increase in carbon-14 incorporation due to irradiation
Kidneys Control	20.2	40
Irradiated	28.3	
Intestinal mucosa Control	35.0	40
Irradiated	49.2	
Liver Control	17.3	106
Irradiated	35.6	
Muscles Control	5.6	
Irradiated	6.5	16*

* In contrast to all other organs investigated in two subsequent experiments, carbon-14 uptake by muscle tissue was not found to be influenced by irradiation.

four hours they observed an exhalation of 87 per cent of the carbon-14 administered. Since our experiments lasted about eight hours, and rapidly growing rats and mice were used, we can expect a still larger percentage exhalation to have taken place. In view of the small percentage of carbon-14 incorporated with the tissues, a decrease of 1 per cent only in the amount exhaled carbon-14 during the experiments can be expected to lead to an increase of 10 per cent, or possibly much more, in the carbon-14 uptake by the tissues. If this interpretation is correct, it should be possible by measuring the increase in the incorporation of carbon-14 in the animal tissue following irradiation or other metabolic interferences to determine small changes produced in the metabolic rate.

References

1. G. HEVESY, *Nature, Lond.* **163**, 869 (1949).
2. R. G. GOULD, F. M. SINEX, I. N. ROSENBERG, A. K. SOLOMON and A.B. HASTINGS, *J. Biol. Chem.* **177**, 295 (1949).

Originally published in *Arkiv för Kemi* **4**, 20 (1951).

81. EFFECT OF X-RAYS ON THE INCORPORATION OF ^{14}C INTO TISSUE FRACTIONS OF THE MOUSE

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MITOTIC arrest is possibly the most conspicuous early effect of ionizing radiation on the living organism. From the view-point of the chemist, cell division is a result of intense and properly correlated cellular synthesis. Correspondingly, one expects the mitotic arrest to be due, at least partly, to an interference with some of these synthetic processes. It was found already several years ago, that irradiation of rat Jensen sarcoma with an X-ray dose of some hundred r interferes with the formation of desoxyribo nucleic acid in the sarcoma⁽¹⁾. In these experiments the rate of incorporation of ^{32}P administered as sodium phosphate into the desoxyribo nucleic acid of the sarcoma of irradiated and control rats was compared. The former were found to take up much less ^{32}P than the controls. While some of this difference was due to the different rate of penetration of ^{32}P into the sarcoma cells, a very appreciable part of it had to be ascribed to an about 50 per cent inhibition of desoxyribo nucleic acid formation. Similar results were obtained from investigations of normal organs of the rat. The blocking effect of radiation on the formation of labelled desoxyribo nucleic acid was already observed $1/2$ hour after irradiation took place.

An objection raised to our results was that the incorporation of ^{32}P with desoxyribo nucleic acid may indicate a re-phosphorylation of the molecule only. An enzymic splitting-off of phosphate groups may be followed by a re-phosphorylation process which in the presence of labelled phosphate, labelled ATP or another phosphate donor results in an incorporation of ^{32}P with the desoxyribo nucleic acid molecule. Though

⁽¹⁾ The bibl. of earlier works, the first paper published being that of H. EULER and G. HEVESY, *Kgl. Danske Videnskab. Selskab Biol. Medd.* **17**, 8 (1942) is to be found in G. HEVESY's contribution to *Adv. of Biol. and Med. Physics* (1948) **1**, 409; see also B. HOLMES, *Brit. Journ. Rad.* **20**, 450 (1947) **22**, 487 (1949); G. HEVESY, *Nature* **163**, 809 (1949); L. S. KELLY and H. B. JONES, *J. Soc. Exp. Biol. Med.* **74**, 493 (1950); H. JONES, *Rep. Oberlin. College Symp.* (1950) in print. A. HOWARD and S. R. PELC, *Ciba Foundation Conference on Isotopes in Biochemistry* p. 147. London (1951).

these objections seemed not justified or to a minor extent only so, — in the growing Jensen sarcoma the rate of additional formation of desoxyribo nucleic acid does not lag much behind the rate of formation of desoxyribo nucleic acid ^{32}P — they induced us to study the effect of irradiation on the incorporation of ^{14}C with the purines of desoxyribo nucleic acid.

In experiments previously described⁽¹⁾ acetate labelled in the carboxyl group was injected to suckling rats irradiated with 880 r and to control suckling rats. The silver purines were secured from the desoxyribo nucleic acid of various organs 4 to 7 hours after injection and their activity was compared. Incorporation of ^{14}C into the purines of the irradiated rats was found to be depressed by 20 to 70 per cent. For the liver fractions the mean values seen in Table 1 were obtained.

The results obtained indicate an arrest caused by irradiation in the formation of purine moiety of desoxyribo nucleic acid as well.

ABRAMS⁽²⁾ investigated recently the effect of irradiation on the incorporation of ^{14}C with purines of the nucleic acids of the rat intestine using glycine — 1 — ^{14}C as source of ^{14}C . He found ^{14}C incorporation with DNA inhibited by 80% and with RNA by 49 %, while the percentage incorporation into proteins was hardly affected.

Urethane is known to interfere with mitotic processes and we can thus expect large doses of urethane to depress nucleic acid formation. In a recent investigation SKIPPER⁽³⁾ administered large doses of urethane (1800 mgm/kg)m and then ^{14}C labelled formate to mice and investigated the incorporation of ^{14}C into the viscera nucleic acids and into the total tissue of various organs. Incorporation of ^{14}C into the viscera nucleic acids was found to be depressed under the effect of urethane to about one half of the value found in controls, thus to a similar extent as the incorporation of acetate ^{14}C into nucleic acid was observed to be reduced under the effect of X-rays (cf. Table 1). Total tissue took up, however, in the urethane injected mice more formate carbon than in the controls. The latter effect is presumably due to a decrease in the sensisivity of the radioactive indicator (higher ^{14}C level) and was formerly observed⁽⁴⁾ in experiments in which following administration of labelled acetate the ^{14}C uptake by tissue was compared in the urethane injected mice and in controls.

The precursor of the nucleic acid ^{14}C may also have a higher activity level in the urethane injected mice than in the controls, but in spite

(1) G. HEVESY, *Nature* **163**, 809 (1949).

(2) R. ABRAMS, *Arch. Biochem.* **30**, 90 (1951).

(3) H. E. SKIPPER, *Texas Rep. Biol. and Med.* **8**, 543 (1950).

(4) G. HEVESY, *Nature* **164**, 1007 (1949); G. HEVESY, R. RUYSSSEN and H. L. BEECKMANS, *Experientia* **7**, 144 (1951).

of the increase in the activity level, due to the strongly inhibiting effect of urethane on nucleic acid formation, the ^{14}C content of the nucleic acid content of the urethane injected mouse is markedly lowered (the last mentioned effect overcompensates the first mentioned one).

As seen in Table 1, irradiation acts in a similar way not only on incorporation of ^{14}C into nucleic acid as does urethane but an increased incorporation of ^{14}C into tissue proteins is observed as well. The last mentioned effect is, however, less pronounced after irradiation and more difficult to reproduce than the effect observed after administration of urethane. In fully grown mice we found a significant increase in ^{14}C incorporation due to irradiation in the brain and possibly into the liver proteins (cf. Table 8).

CHANGE IN THE SENSITIVITY OF THE RADIOACTIVE INDICATOR WITH TIME

The change in the sensitivity of the radioactive indicator with time plays an important role in many applications of radioactive tracers, this being especially the case in the study of the metabolism of acetate and other rapidly metabolizing carbon compounds.

Some years ago, MARINELLI⁽¹⁾ studied the incorporation of ^{32}P into the bones a few days after irradiating one leg of the rabbit with a 3000 r dose, while the other legs were protected. He found that if ^{32}P was injected into the animal several days after irradiation of one of the legs, the irradiated leg contained only about 70 per cent of the ^{32}P content of the non-irradiated leg. The inhibition of radiophosphate uptake by the irradiated leg leads to a higher activity level of the plasma inorganic P and correspondingly to a higher ^{32}P uptake by the phosphorus compounds of the soft tissues. We meet thus indirect radiation effects. Radiolesion of the bone may reflect itself in the rate of incorporation of ^{32}P into liver phosphatides and many other compounds. It is not the turnover rate itself of the liver phosphatides which is influenced, but the sensitivity of the registration of the turnover.

The application of ^{14}C as an indicator, especially in the form of rapidly metabolizing compounds such as acetate, may reveal a great variety of indirect radiation effects.

If we inhibit by irradiation ^{14}C labelled protein or phosphatide formation in the liver, the protein resp. phosphatide content of the plasma will decrease and all steps in which these compounds participate will be influenced as well.

⁽¹⁾ L. D. MARINELLI, *Radiology* **37**, 169 (1941).

If irradiation blocks the incorporation of acetate carbon into fatty acids or cholesterol, more ^{14}C may take its way through other metabolic pathways. Such an interference will lead to an increased ^{14}C level of the CO_2 of body fluids, the result of this increase being, for example, an enhanced ^{14}C incorporation in liver or muscle glycogen. A decrease in the amount of ^{14}C trapped in metabolic pools of Krebs' cycle due to irradiation lesions will act in the same way. This increase in the ^{14}C content of glycogen may not be due to a larger glycogen production, but to a lowered sensitivity of ^{14}C as an indicator. A radiation lesion produced in the liver may thus be responsible for an enhanced ^{14}C incorporation into a muscle fraction.

In a similar way, interference with the conversion of acetate into acetoacetate in the liver will interfere with the ^{14}C incorporation into muscle fractions, most of the muscle acetoacetate being presumably carried into that tissue from the liver. In this case, we interfere not only with the sensitivity of the indicator of processes taking place in the muscle, but even with the turnover rate.

The injected labelled acetate is diluted with endogenous acetate formed by catabolism of fatty acids and other compounds. If irradiation interferes with the catabolic processes or with the rate of interchange between intra- and extra hepatic fatty acids, but does not influence, or influences to a minor extent only the incorporation of acetate into fatty acids, the activity level of body acetate will be increased and we observe an increased ^{14}C incorporation in fatty acid fraction or protein samples, for example, which is due not to an increased turnover rate, but to a decrease in the sensitivity of the indicator. More ^{14}C is incorporated with the tissue fraction, but the amount of ^{12}C renewed may be practically the same, and vice versa.

While a change in the sensitivity of the radioactive indicator may be responsible for the change in the ^{14}C uptake observed, this is far from always the case. Irradiation may divert ^{14}C present in intermediary compounds of acetate metabolism from oxidation via the Krebs' cycle. Such diversions were repeatedly observed, for example, in experiments with liver slices⁽¹⁾. Addition of pyruvate, citrate, α -ketoglutarate or malonate was found to diminish the output of $^{14}\text{CO}_2$, while an increased incorporation of ^{14}C into α -ketoglutarate and other compounds was found to take place. Furthermore ALTMAN *et al.*⁽²⁾, when investigating marrow homogenates of irradiated and of control rabbits incubated in the presence of α - ^{14}C -acetate found a markedly increased incorporation

(1) A. B. PARDEE, C. HEIDELBERGER and V. R. POTTER, *J. Biol. Chem.* **186**, 625 (1950).

(2) K. I. ALTMAN, J. E. RICHMOND and K. SALOMON, *Biochem. et Biophys. Acta* **7**, 460 (1950).

of ^{14}C into the fatty acid of homogenates of the marrow of irradiated rabbits.

The application of ^{14}C as indicator may thus reveal radiation lesions both in the irradiated organ and other organs, due either to changes in the turnover rate or to the change in sensitivity of the indicator, only.

SOME ASPECTS OF ACETATE METABOLISM

To the daily diet of the rat we can add a constant amount of acetate labelled in the carboxyl group and obtain an almost constant activity level of the body acetate. This activity level is measured by feeding phenylbutyric acid to the rat, isolating the excreted phenylbutyric acetate, and determining its ^{14}C content.

By comparing at the end of the experiment, the ^{14}C content of the fatty acid carbon of the liver with the corresponding value of the urinary phenylbutyric acetate, as shown by RITTENBERG and BLOCH,⁽¹⁾ the rate of the liver fatty acids can be calculated.

In a recent investigation, PIHL *et al.*⁽²⁾ have shown that prolonged daily feeding of a constant amount of labelled acetate does not lead to a constant but to an increasing activity level of the body acetate with time. This contrasts with the results obtained when feeding heavy water which results in a practically constant deuterium level of the body fluids. Though intake of heavy water is followed by the formation of deuteriated body constituents which, in turn, are catabolized and form deuteriated water, in view of the large water content of the body (about 70 per cent) these additional amounts of catabolic heavy water do not much influence the deuterium content of the body water. In a similar way, the catabolism of ^{14}C labelled fatty acids and other compounds ensues in the formation of labelled acetate. In view of the low (much less than 1 per cent) acetate content of the body fluids the additional catabolic labelled acetate can not, however, be disregarded and correspondingly, in experiments of many days, through which a constant amount of labelled acetate is fed, a quite marked increase in the activity level of body fluid acetate takes place.

A very different behaviour of the labelled acetate is observed when it is administered, for example by intraperitoneal injection, at the start of the experiment⁽³⁾. The catabolism of the administered acetate leads to a large extent to the formation of $^{14}\text{CO}_2$, which is exhaled. In the rat

(1) D. J. RITTENBERG and K. BLOCH, *Biol. Chem.* **160**, 417 (1945).

(2) A. PIHL, K. BLOCH and H. S. ANKER, *J. Biol. Chem.* **133**, 441 (1950).

(3) cf. G. HEVESY, R. RUYSSSEN and M. L. BEECKMANS, *Experientia* **7**, 144 (1951).

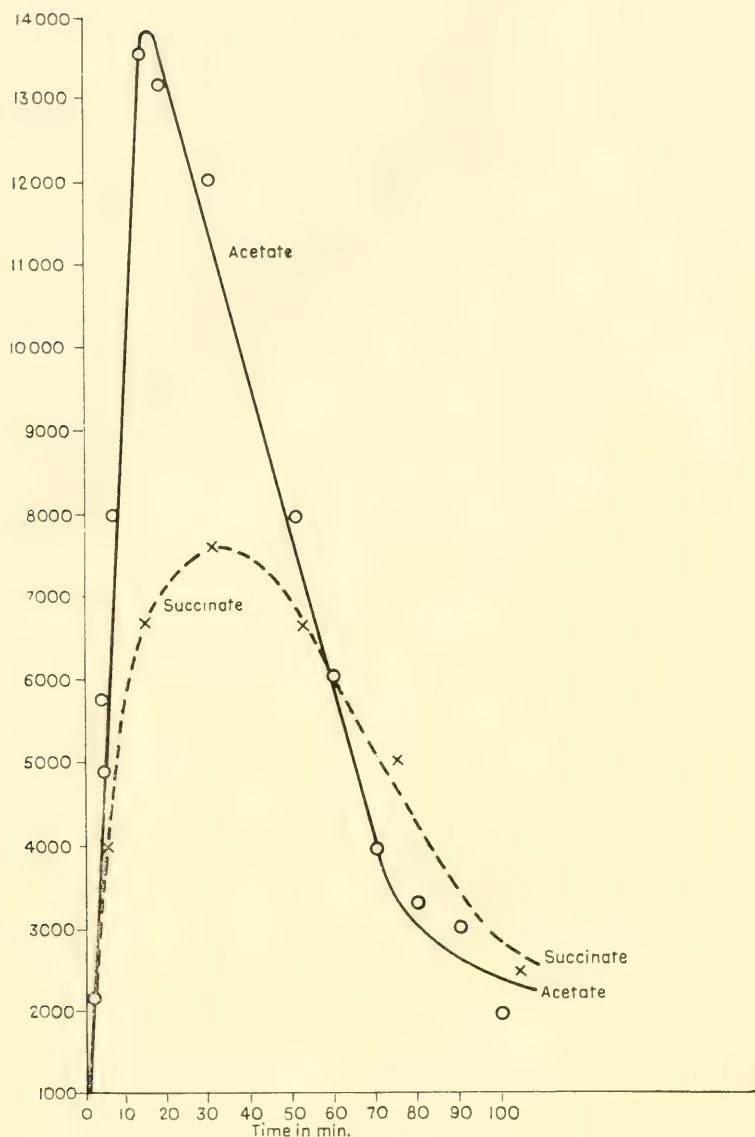


FIG. 1. Change in specific activity of the expiratory carbon dioxide following injection to rats of labelled acetate and succinate, respectively (GOULD et al.)⁽¹⁾

the specific activity of the expiratory CO_2 increases in the course of the first 20 minutes (in the mouse during a shorter interval), absorption, distribution and metabolism of acetate taking some time. $^{14}\text{CO}_2$ forma-

⁽¹⁾ R. G. GOULD, F. M. SINEX, I. N. ROSENBERG, A. K. SOLOMON and A. P. HASTINGS, *J. Biol. Chem.* **177**, 295 (1949).

tion being soon exhausted or almost exhausted while endogenous $^{12}\text{CO}_2$ is constantly produced, the specific activity of the respiratory carbon dioxide is bound to decrease markedly with time in the later phase of the experiment.

After the lapse of 50 minutes, the specific activity of the exhaled carbon dioxide is only half of the value observed after the lapse of 20 minutes.

If the injected labelled carbon compound is metabolized at a less rapid rate than acetate or if we interfere with the normal metabolic rate of acetate in the early phase of the experiment, the increase in the $^{14}\text{CO}_2$ content of the respiratory carbon dioxide will take place at a slower rate, than in that of normally metabolizing acetate and so will the decrease in the specific activity occurring in the later stage of the experiment. This is the case when the slower metabolizing labelled succinate is administered to rats. Figure 1 which is plotted by making use of the data of GOULD and assoc.⁽¹⁾ demonstrates the change in specific activity of the expiratory carbon dioxide following injection of labelled acetate and succinate respectively. The results obtained by the above mentioned authors, clearly demonstrate the lower metabolic rate of succinate. The velocity constant of the conversion of acetate carboxyl carbon and succinate carboxyl carbon into carbon dioxide is found by the above mentioned authors to be 0.043 min^{-1} and 0.028 min^{-1} respectively.

If the irradiation influences the metabolic rate of acetate the produced change will reflect itself in a change in the slope of the time-specific activity curve of the exhaled carbon dioxide, as the amount of $^{14}\text{CO}_2$ contributed to the CO_2 exhaled in the time unit will now differ from that contributed by the controls. Catabolic changes in which labelled acetate does not participate, or participates to a minor extent only, may also influence the slope of the time-specific activity curves of exhaled carbon dioxide. The amount of $^{12}\text{CO}_2$ to which a given quantity of $^{14}\text{CO}_2$ is to be admixed, will now be different. The change in the time-specific activity curves will reflect itself in activity figures for all tissue fractions into which CO_2 is incorporated. A metabolic interference which leads to an increase in the $^{14}\text{CO}_2$ content of the liver CO_2 (bicarbonate), so far as it does not influence glycogen turnover, will result in higher activity figures of glycogen. In this statement we disregard possible differences in the ^{14}C incorporation due to the fact that some ^{14}C enters glycogen by another way than through circulating CO_2 .

An increased ^{14}C content of the liver glycogen after irradiation may be due to an increased turnover rate of glycogen. If this leads to

(1) R. G. GOULD, F. M. SINEX, I. N. ROSENBERG, A. K. SOLOMON and A. B. HASTINGS, *J. Biol. Chem.* **177**, 295 (1949).

an increase in the glycogen content of the liver it will be revealed by analytical data⁽²⁾. If the glycogen content remains constant during the experiment the increase in ^{14}C content may be due to an increased rate

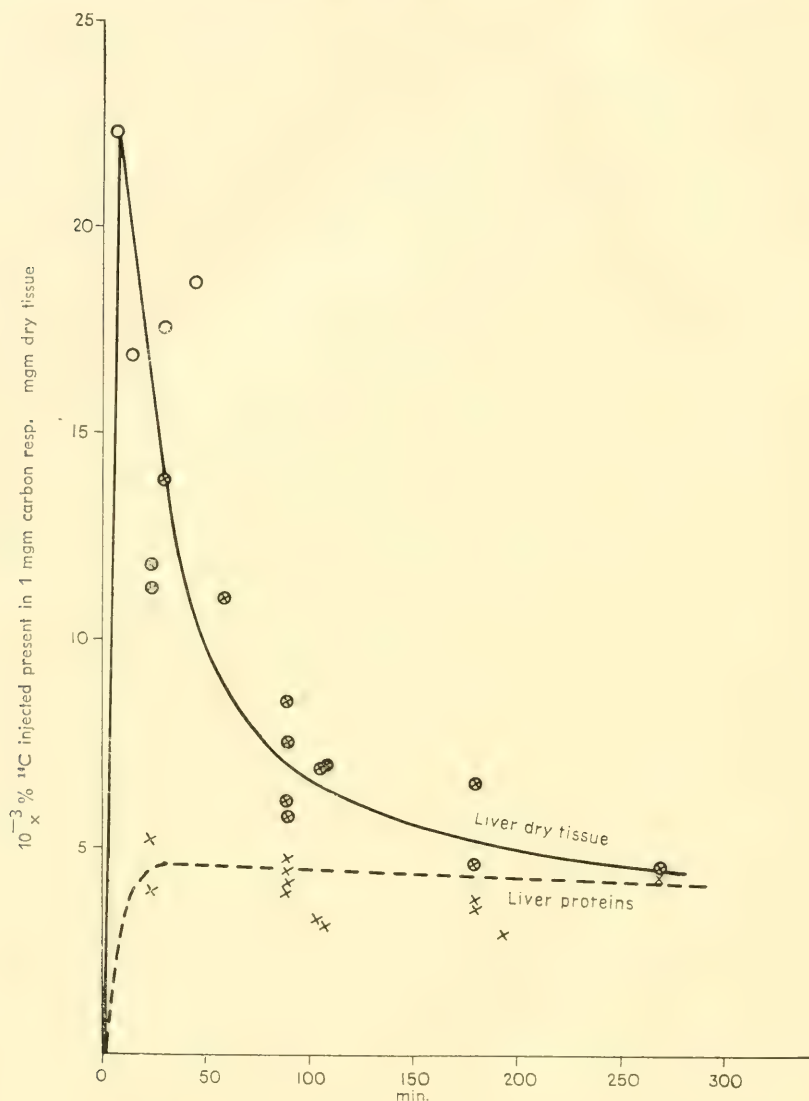


FIG. 2. Change of the ^{14}C content of liver dry tissue and liver proteins of mice injected with $\text{NaCH}_3^{14}\text{CO}_2$ with time. Each point represents the value obtained for 10 or more pooled livers. Values obtained for mice of different ages and races were plotted solely to demonstrate the great difference in the change of the ^{14}C content of the liver tissue and liver proteins with time.

² N. NORTH and L. F. NIMS, *Fed. Proc.* **3**, 119 (1949); M. H. ROSS and I. O. ELEY, *J. cell. comp. Physiol.* **37**, 163 (1951).

of formation of labelled glycogen compensated by an increased catabolism of almost inactive glycogen. An increased ^{14}C content of the liver glycogen after irradiation may, however, be due to a very different reason as well, to a change in the sensitivity of $^{14}\text{CO}_2$ as an indicator of $^{12}\text{CO}_2$. If that to be the case, then if $^{14}\text{CO}_2$ formation from acetate is slowed down in the first phase of the experiment (during the first minutes), the $^{14}\text{CO}_2$ content of the liver bicarbonate will be lower in the irradiated animals than in the controls. Soon, however, the opposite will be the case and thus a larger incorporation of ^{14}C into glycogen will be observed.

If it is not the specific activity of the carbon dioxide which is influenced by irradiation, but it is the turnover rate of glycogen which is enhanced, we should find in all phases of the experiment more ^{14}C in the glycogen fraction. If, however, an increase in the ^{14}C content of the carbon dioxide is responsible for the changed ^{14}C uptake by the glycogen, we should observe an increased ^{14}C incorporation into glycogen in the first phase of the experiment and a decreased one in the later phase. "Time experiments" may thus supply important information about the metabolic changes produced by irradiation.

Much of the respiratory CO_2 is the product of muscular metabolism. Changes in the specific activity of carbon dioxide produced in an organ with a minor contribution to the total CO_2 production of the organism may, therefore, not suffice to become clearly visible when comparing the specific activities of CO_2 of irradiated animals with that of controls. Though such comparisons are of importance, they often are less revealing than investigations into the effect of irradiation on the ^{14}C content of tissue fractions.

We discussed above the possible effects of irradiation on the specific activity of catabolic carbon dioxide. We shall now consider the result of interference with the rate of formation of another product of acetate metabolism with that of fatty acids.

Part of the acetate carbon which reaches the liver is rapidly incorporated into the fatty acids of the liver of the mouse. After the lapse of about 20 minutes the fatty acids show a maximum ^{14}C content, half of that value being observed only after the lapse of further 40 minutes. Many of the newly formed fatty acid molecules are soon leaving the liver by catabolic processes or exodus and, as in the mean time the activity level of the acetate and other fatty acid precursors is lowered, the disappeared active fatty acid molecules are replaced by less active ones. The marked decline in the activity level of acetate with time is, as already mentioned, due to the fact that the injected labelled acetate is rapidly metabolized, while the diluting non-radioactive endogenous acetate due to incessant new-formation remains at a constant concentration level.

In the liver of mice killed at different intervals after injecting labelled acetate, an acceleration of the rate of conversion of acetate into fatty acids can be expected to lead in the first minutes to an increased ^{14}C content of the fatty acids. However, when a maximum activity is reached (cf. right part of the curves in Fig. 3), due to the now increased rate of disappearance of the administered labelled acetate with time a more

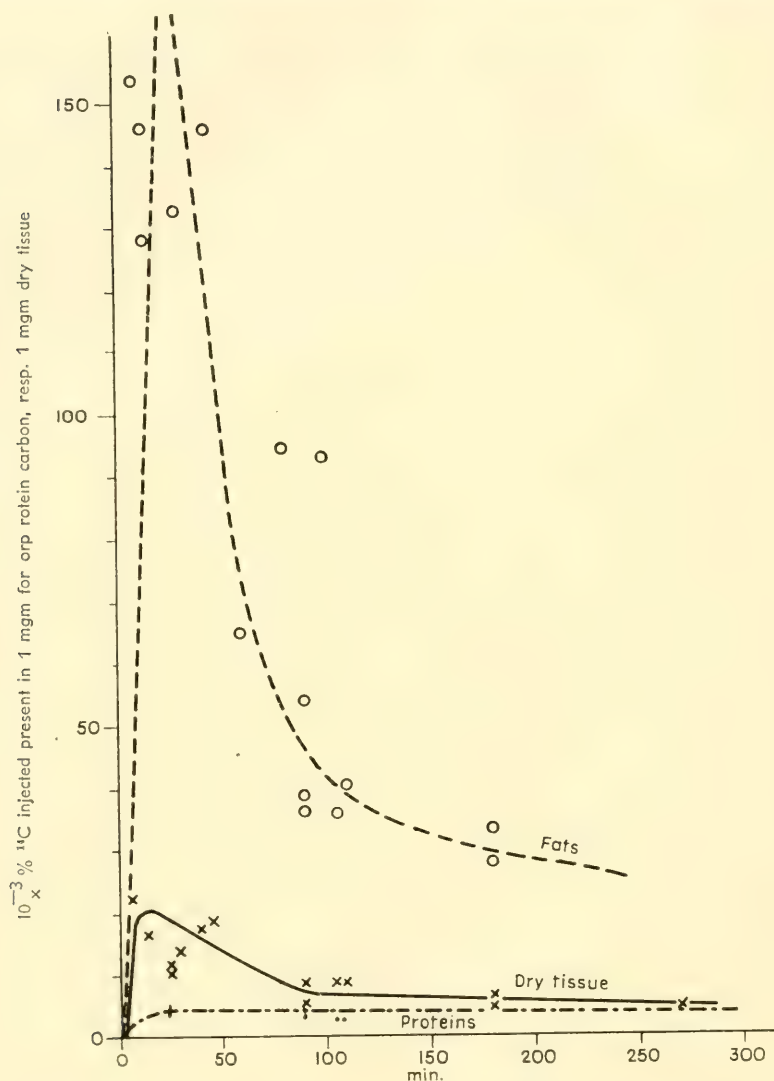


FIG. 3. Change of the ^{14}C content of liver fats, liver total tissue and liver proteins of mice injected with $\text{NaCH}_3^{14}\text{CO}_2$ with time. Each point represents the value obtained for 10 or more pooled livers. Values obtained for mice of different ages and races were plotted solely to demonstrate the great difference in the change of the ^{14}C content of liver fats and liver proteins with time.

rapid decrease in the specific activity of the body acetate will take place and correspondingly the active fatty acid molecules will be more rapidly replaced by less active ones than in the absence of a metabolic accelerator. Thus, when a maximum fatty acid activity is reached the specific activity figures will decline more rapidly than they do in the case of the normally metabolizing liver and vice versa.

The rapid decline a few minutes only after the administration of the labelled acetate in the activity of the liver fats due to a large extent to the activity of fatty acids, is demonstrated in Figs. 2 and 3. These are also showing the very different behaviour of the liver proteins and also that the change in the activity of the total tissue with time much resembles the change in the activity of fats. The data were obtained by investigating mice of different race and age and were plotted with the sole reason of demonstrating the spectacular decline of the activity of liver fat and total tissue in contrast to that of proteins in the first hours of the experiment.

Administration of cyclopentylhexane, which is a metabolic accelerator, was found to have the above described effect on the time-specific activity curves of liver fats⁽¹⁾.

Metabolic depressors can be expected to act in the opposite direction. Their presence may slow down the rate of incorporation of ^{14}C into fatty acids, but the activity level of the fatty acids will now be longer maintained, since the replacement of the active fatty acid molecules by less active ones takes place at a slower rate. Furthermore, a decreased catabolic rate of glucose, for example, will lead to a reduced formation of fatty acids, which results (these fatty acids being almost inactive) in a reduced dilution of the labelled acetate leading in turn to a decrease in the sensitivity of ^{14}C as an indicator of fatty acids.

A comparison of the ^{14}C content of the total fat or of the phosphatides of mice injected with urethane with that of the controls, brings out these effects of a metabolic depressor⁽²⁾.

Metabolic activators and depressors have — as seen above — a marked influence on the incorporation of ^{14}C into tissue fractions. If such effects were produced by irradiation with ionizing radiation, we should observe significant changes in the rate of incorporation of ^{14}C into tissue, fractions and possibly in the specific activity and even in the amount of exhaled CO_2 .

When making the above remarks, we have to envisage the existence of several competitive paths which acetate metabolism follows, that two

⁽¹⁾ M. L. BEECKMANS, M. CASIER and G. HEVESY, *Arch. Int. Pharmacodyn.*, **80**, 33 (1950).

⁽²⁾ G. HEVESY, *Nature* **164**, 1007 (1949); G. HEVESY, R. RUYSEN and M. L. BEECKMANS, *Experimentia* **7**, 317 (1951); H. A. SKIPPER, *Texas Rep. Biol. and Med.* **8**, 543 (1950).

carbon fragments find their way into cholesterol, steroid hormones, hemin and fatty acids on one side and into citric acid, glutamic acid, proline, arginine on the other side. The suppression of any of these paths may lead to an increased ^{14}C incorporation in the metabolic products involved in the remaining paths.

EXPERIMENTAL

The X-ray tube applied, was run with 180 kW and 7 milliamp. The distance between the anticathode and the surface of the paperbox in which the mice were placed was 40 cm.

Adult mice were irradiated for 3 min. with a dose of 295 r per min. The mice had access to food all through the experiment. They were injected intraperitoneally or, in some cases, subcutaneously mostly with 0.1 ml of physiological sodium chloride solution containing in the carboxyl group labelled sodium acetate of an activity of 2 to 6 microcuries. In some experiments, injection with acetate took place immediately after irradiation, in others up to several hours later. At least 10 mice were irradiated in each experiment. The mice were killed by decapitation. In most experiments blood plasma, liver, kidneys, intestinal mucosa, muscles and brain, in some cases femur, lungs and spleen were secured as well. The intestines were carefully rinsed before securing the mucosa. An aliquot of the organs was dried and the activity of the samples of the pooled organs of irradiated mice and that of the controls compared. From another aliquot of the organs the total fats were obtained by extracting the ground organs with a boiling mixture of ether-alcohol (1 : 3) for 3 hr. The filtrate obtained was evaporated *in vacuo* and the residue extracted with petroleum-ether. The evaporation of the petroleum-ether gave the total fats. In our later experiments, these were purified with colloidal iron following the procedure of FOLCH and VAN SLYKE as described below.

In order to obtain the phosphatides, the total fats were dissolved in petroleum-ether and precipitated with twice the amount of cold acetone and 5 drops of an alcoholic solution of 4.5% MgCl_2 , precipitation being completed by standing for 2 hr in the refrigerator. The phosphatides were centrifuged. The precipitate dissolved in petroleum-ether was reprecipitated in the same way. After centrifugation the precipitate dissolved in petroleum-ether was washed with distilled water. Evaporation of the petroleum-ether gave the phosphatides.

The supernatants from the precipitation of the phosphatides were combined and evaporated. The residue was saponified by boiling for 8 hr with 10 cc. of an aqueous solution of 40% KOH and 20 cc. alcohol. After saponification the solution was extracted several times with petroleum-ether. To the petroleum-ether solution after being brought to a small volume twice its volume of a 0.5% solution of digitonin in 80% alcohol was added. The quantity of digitonin was about 5 times the amount of cholesterol expected. The precipitated cholesterol was first washed with 80% alcohol to eliminate the excess of digitonin, then with a mixture of acetone-ether (1 : 2) and finally with ether; it was dried at 37° .⁽¹⁾

When investigating the effect of irradiation on cholesterol formation, we compared the activity of cholesterol digitonin samples. The presence of digitonin reduced the activity of cholesterol to about to 1/4 of its value, conversion of cho-

⁽¹⁾ P. A. SVERE, I. L. CHAIKOFF and W. G. DAUBEN, *J. Biol. Chem.* **176**, 829 (1948).

lesterol carbon into barium carbonate would have resulted in a still greater reduction of the activity figures.

The fat-free tissue, after being repeatedly washed with water was repeatedly treated at 90°C for 10 min. with 5% trichloroacetic acid to obtain the raw proteins. These were abundantly rinsed with water, then dried by washing with alcohol and ether. Subsequently the activity of the protein samples was compared.

We purified in our later experiments the total fat samples with colloidal iron⁽¹⁾. In the case of liver fats such a purification leads to an increase in the activity figures. In experiments taking 3 hours, an average increase of 11 per cent was observed, while Dr. BEECKMANS working in this laboratory found in experiments taking 15 minutes a 7 per cent average increase. Treatment with colloidal iron removes thus less active constituents of the liver fats, possibly urea, amino acids and other impurities. Some phosphatides are removed by their treatment as well. In experiments taking not more than a few hours, the activity of phosphatides is less than the activity of the total fats of the same weight, the ratio of the activity of 1 mgm total fat and 1 mgm phosphatide being about 1.3. Some removal of less active phosphatides in the course of the purification process with colloidal iron will thus also lead to an increase in the activity of the total fats of the liver.

Purification with iron has very different effects on brain fats. After purification their activity decreases with 11 per cent in experiments taking 3 hours. More active fractions are thus removed by this process. The decrease in the activity figures due to purification is much larger in experiments of short duration. In experiments taking 15 min. only, treatment with colloidal iron removes $3/4$ of the ^{14}C content of the total fats, 45 % of that of the muscle fat and $1/3$ of the intestinal mucosa.

The dry liver weight of fed mice weighing 22.7 gm (mean value of a few hundred animals) was 0.31 gm. Liver dry weight amounted to 28.2 % of its fresh weight. We found the fat content of dry liver tissue = 21.7%, that of fresh tissue = 7.4%.⁽²⁾ In the liver of fed 15 gm mice STETTEN and GRAHL⁽³⁾ found a fatty acid content of 4.5%. From the above data it follows that 61% of the liver fats is made out of fatty acids. 17.1% of the weight of the fresh liver was made out of proteins.

When comparing the activity of the dry total tissue or protein samples secured from irradiated mice and from controls, 50 mgm of each to the dried samples we placed in an aluminium dish of 1.2 cm diameter and the activity of the samples was compared. The small size counters used had a mica window of about 2 mgm per cm^2 and a background of about 3 counts per minute.

The fat samples, the activity of which was appreciably larger than that of the total dry tissue or protein samples were placed in Ottesen's micro dishes, which proved to be very suitable for this purpose. The counts measured for an infinitely thick fat sample placed in a dish of 1.2 cm diameter made out 4.8 times the number of counts measured when the infinitely thick fat sample was placed in a micro dish.

In some cases we burnt our samples and compared their activity after converting their carbon into BaCO_3 . In view of the work involved in this procedure (we measured the activity of a very great number of samples) and also because the low carbon content of BaCO_3 we preferred to compare the activity of our samples without converting them into barium carbonate. Total liver fat samples, for example, which were found to contain 65.2 per cent carbon, while the carbon

⁽¹⁾ J. FOLCH and D. VAN SLYKE, *Proc. Exp. Biol. Med.* **41**, 514 (1939).

⁽²⁾ H. C. HODGE, P. L. MACLACHAN, W. R. BLOOR, E. WELCH, S. L. KORNBERG and M. FALKENHEIM state a fat content of 7%. *J. Biol. Chem.* **67**, 137 (1948).

⁽³⁾ D. STETTEN Jr. and G. F. GRAHL, *J. Biol. Chem.* **148**, 509 (1943).

content of barium carbonate amounts to 6.1 per cent only. A burning of a fat sample and the conversion of the carbon obtained into barium carbonate leads thus to a product the activity of which makes out 9.1 per cent only of the activity of fat of the same weight, this figure being increased to 11 when taking into account the reflecting power of barium for the β -rays emitted by the sample.

RESULTS

Some of the results obtained when comparing the ^{14}C content of irradiated mice and that of controls is seen in the following tables.

TABLE 1. — PERCENTAGE INHIBITION BY AN X-RAY DOSE OF 880 R OF THE INCORPORATION OF ^{14}C INTO PURINES OF DESOXYRIBONUCLEIC ACID PREPARED FROM THE LIVER OF SUCKLING RATS

Fraction	Mean value obtained from 55 animals Percentage Change in the Incorporation of ^{14}C due to the Effect of Irradiation
Purines	-44
Proteins	+27

TABLE 2. — PERCENTAGE CARBON CONTENT ASCERTAINED IN SOME OF OUR TISSUE SAMPLES

Liver fat	65.2
Liver protein	48.8
Intestinal mucosa fat	64.0
Intestinal mucosa protein	47.3

20 mice weighing 21–27 gm were irradiated. Half of these — having an average weight of 23.5 gm each — were injected immediately, half after the lapse of 6 hr with 0.15 ml labelled acetate having an activity of 49,200 counts per min. A third group of 10 mice — aggregate weight 238 gm — was injected without being irradiated. All mice were killed 1.5 hours after the injection.

We arrived at the figure 49,200 stated above by dissolving a known aliquot of the injected acetate in 50 mgm of a concentrated sodium acetate solution having the same vapour pressure as the atmosphere at the time of measurement and measuring the activity of the solution. All activity data of the tables 3–6, which contain some of the results obtained, are such of a 50 mg n sample. We did not correct for the different reflecting power of the acetate solution, fat samples and so on. The correction to be employed is very restricted. Furthermore, we were interested primarily

TABLE 3. — EFFECTS OF IRRADIATION ON THE INCORPORATION OF ^{14}C INTO TISSUE FRACTIONS*Dry Tissue*

Organ		Counts/min. per mgm tissue	Percent of activity injected present in 1 mgm tissue $\times 10^3$
Liver	Roentgen I*	3.800	9.10
	Control	3.545	8.50
	Roentgen II**	3.555	8.52
Intestinal mucosa	R I	4.570	10.95
	C	3.930	9.43
	R II	4.110	9.85
Muscles	R I	0.541	1.298
	C	0.430	1.030
	R II	0.434	1.041
Kidney	R I	1.811	4.340
	C	1.554	3.725
	R II	1.571	3.770
Brain	R I	0.769	1.841
	C	0.460	1.101
	R II	0.526	1.261
Plasma	R I	3.845	9.21
	C	3.120	7.48
	R II	3.375	8.09
Bone	R I	0.462	1.108
	C	0.429	1.130
	R II	0.408	0.979

Organ		Per cent of activity injected present in 1 mgm carbon $\times 10^3$	Organ		Percent of activity injected present in 1 mgm carbon $\times 10^3$
<i>Total fat</i>			<i>Proteins</i>		
Liver	Roentgen I*	45.4	Liver	Roentgen I*	5.20
	Control	38.6		Control	4.32
	Roeng. II**	53.4		Roengt. II**	4.46
Intestinal mucosa ...	R I	38.4	Intestinal mucosa ...	R I	18.16
	C	32.7		C	18.11
	R II	28.9		R II	17.40
Muscles	R I	3.63	Muscles	R I	0.509
	C	5.06		C	0.606
	R II	3.09		R II	0.597
Kidneys	R I	14.9	Kidney	R I	3.90
	C	14.0		C	3.99
	R II	13.0		R II	3.98
Brain	R I	4.55	Brain	R I	0.528
	C	3.36		C	0.509
	R II	3.67		R II	0.615

* Roentgen I = injected 6 hr after irradiation.

** Roentgen II = injected immediately after irradiation.



TABLE 4.—20 GROWING MICE WEIGHING 9—17 GM WERE IRRADIATED. HALF OF THESE (AGGREGATE WEIGHT 130 GM) INJECTED IMMEDIATELY, THE OTHER HALF AFTER THE LAPSE OF 6 HOURS. A THIRD GROUP (10 MICE) INJECTED WITHOUT BEING IRRADIATED. EACH MOUSE WAS INJECTED WITH 301,000 COUNTS PER MIN.

Dry Tissue

Organ		Counts/min. per mgm tissue	Percent of activity in- jected present in 1 mgm tissue $\times 10^3$
Liver	Roentgen I*	3.35	7.23
	Control	3.47	7.49
	Roentgen II**	2.68	5.78
Intestinal mucosa	R I	3.65	7.88
	C	4.54	9.81
	R II	4.73	10.21
Muscles	R I	0.508	1.098
	C	0.468	1.010
	R II	0.510	1.100
Brain	R I	0.458	0.990
	C	0.442	0.912
	R II	0.698	1.507
Lungs	R I	1.296	2.80
	C	1.290	2.79
	R II	1.362	2.95
Plasma	R I	1.740	3.76
	C	1.800	3.89
	R II	1.995	4.31

* Injected 6 hr after irradiation.

** Injected immediately after irradiation.

Total fat and protein

Organ		Percent of activity injected present in 1 mgm carbon $\times 10^3$	
		Total fat	Protein
Liver	Roentgen I	53.9	3.92
	Control	54.1	4.15
	Roentgen II	33.8	3.97
Intestinal mucosa	R I	32.3	12.80
	C	39.4	16.75
	R II	38.9	18.76
Muscles	R I	9.58	0.572
	C	4.74	0.605
	R II	3.47	0.543
Brain	R I	3.91	0.446
	C	2.94	0.482
	R II	4.69	0.615
Lungs	R I	19.9	1.570
	C	15.7	1.795
	R II	15.5	2.075
Plasma	R I		5.21
	C		5.77
	R II		6.17

TABLE 5. — 10 MICE WEIGHING 11–17 GM WERE IRRADIATED AND AFTER THE LAPSE OF 24.5 HOURS INJECTED. THE SAME NUMBER OF MICE INJECTED WITHOUT BEING IRRADIATED. AGGREGATE WEIGHT OF THE 2 GROUPS 140 AND 138 GM EACH MOUSE INJECTED WITH 51,000 COUNTS/PER MIN. KILLED 90 MINUTES AFTER INJECTION

Dry tissue

Organ		Counts/min per mgm tissue	Percent of activity in- jected in 1 mgm tissue $\times 10^3$
Liver	R	5.12	6.97
	C	4.46	6.07
Intestinal mucosa .	R	7.39	10.06
	C	7.79	10.86
Muscles	R	1.14	1.56
	C	1.02	1.39
Brain	R	1.59	2.16
	C	1.23	1.67
Lungs	R	2.67	3.64
	C	2.13	2.90
Plasma	R	5.26	7.15
	C	3.63	4.94
Plasma extracted .. with ether-alcohol..	R	4.07	5.54
	C	2.90	3.96

Total fat and protein

Organ		Per cent of activity injected present in 1 mgm carbon $\times 10^3$	
		Total fat	Protein
Liver	R	37.2	6.66
	C	40.9	4.62
Intestinal mucosa .	R	29.3	20.05
	C	23.3	24.40
Muscles	R	6.70	1.25
	C	7.65	1.01
Brain	R	6.15	1.17
	C	7.20	0.96
Lungs	R	15.3	2.89
	C	20.9	2.51
Plasma	R		10.68
	C		8.38

in a difference of the activity of samples of identical composition, for example of that of liver proteins extracted from irradiated and non-irradiated mice and not in an accurate determination of the retention figures.

TABLE 6. — 10 MICE WEIGHING 18—23 GM WERE IRRADIATED AND AFTER THE LAPSE OF 24 HOURS INJECTED. THE SAME NUMBER OF MICE INJECTED WITHOUT BEING IRRADIATED. AGGREGATE WEIGHT OF EACH GROUP 201 GM. EACH MOUSE INJECTED WITH 38,600 COUNTS. KILLED 105 MINUTES AFTER INJECTION

Dry tissue

Organ		Counts/min per mgm tissue	Per cent of activity in- jected present in 1 mgm tissue $\times 10^3$
Liver	R	2.455	6.37
	C	2.665	6.91
Intestinal mucosa	R	3.080	7.99
	C	3.530	9.14
Muscles	R	0.387	1.00
	C	0.263	0.68
Kidney	R	1.150	2.98
	C	0.910	2.34
Brain	R	0.328	0.845
	C	0.280	0.724
Plasma	R	2.810	7.28
	C	2.405	6.23

Total fat and protein

Organ		Per cent of activity in 1 mgm carbon $\times 10^3$	
		Fat	Protein
Liver	R	36.0	3.60
	C	31.2	3.31
Intestinal mucosa	R	29.8	14.72
	C	22.0	14.89
Muscles	R	2.24	0.508
	C	4.64	0.564
Kidney	R	6.42	2.82
	C	9.08	2.49
Brain	R	1.89	0.458
	C	2.39	0.356

The above figures demonstrate the very marked renewal rate of fat carbon especially in the liver and intestinal mucosa, while from the protein fractions those of the intestinal mucosa are far the most active ones.

EXPERIMENTS OF VERY SHORT DURATION

As we wished to obtain information on the possible effect of irradiation on the rate of resorption of intraperitoneally injected acetate, we killed the mice in a few experiments 5 to 15 minutes only after the injection took place. 0.1 ml of saline containing about 10^5 counts/min of labelled acetate was injected to groups of 4 mice. In contrast to all other experiments, in these experiments of very short duration food, not, however, water, was withheld from the mice after irradiation.

As seen in Table 6a the ^{14}C content of the blood of the irradiated and non-irradiated mice does not differ much. By adding to half of the blood secured 0.5 ml of 2-n HCl and drying the blood *in vacuo*, a process which was repeated, we removed the carbonate content of the blood. In the 5 min experiments this procedure lead to a diminution of the blood activity by 25 to 27 per cent for the non-irradiated blood and by 28 to 45 per cent for the irradiated blood.

The figures of Table 6a do not indicate a depressed resorption after irradiation. Irradiation depressed in these experiments ^{14}C incorporation into liver fats, in the 5, 15 and 30 min experiments by 56, 67 and 45 per cent respectively. Liver fat figures show as in the other experiments described very fluctuating values.

EFFECT OF IRRADIATION ON SUCKLING RATS

The summary of a few experiments carried out with 7 to 14 day old rats which had access to food, except for the 3 min which irradiation has taken, is seen in Table 7.

In an experiment with 34 suckling rats, weighing 40 gm, the animals had no access to food for the 4 1/4 hr which elapsed between injection with acetate and the killing of the rats. Injection took place after irra-

TABLE 6a. — EFFECT OF IRRADIATION ON MICE KILLED A FEW MINUTES AFTER ADMINISTRATION OF LABELLED ACETATE

Fraction	Counts/min./100 mgm dry sample					
	Time of experiment					
	5 min		15 min		30 min	
Blood control	35.7;	31.3	35.5;	36.6;	32.9	34.6
Blood irr.	35.4;	39.0	35.0;	36.0;	29.9	29.9
Liver fat control	508;	699	210;	786;	803	473
Liver fat irr.	167;	306	188;	682;	545	460
Liver prot. control....	—	18.2	—	42.7	—	52.7
Liver prot. irr.	—	10.2	—	42.4	—	58.5

TABLE 7. — PERCENTAGE CHANGE IN THE INCORPORATION OF ^{14}C INTO PROTEIN FRACTION OF SUCKLING RATS DUE TO IRRADIATION WITH 880 R.

Number of rats	31	27	28
Liver	+ 27	+ 20	+ 21
Intestinal mucosa	+ 9	+ 3	—
Kidney	— 2	— 5	— 20
Muscle	0	— 3	+ 5

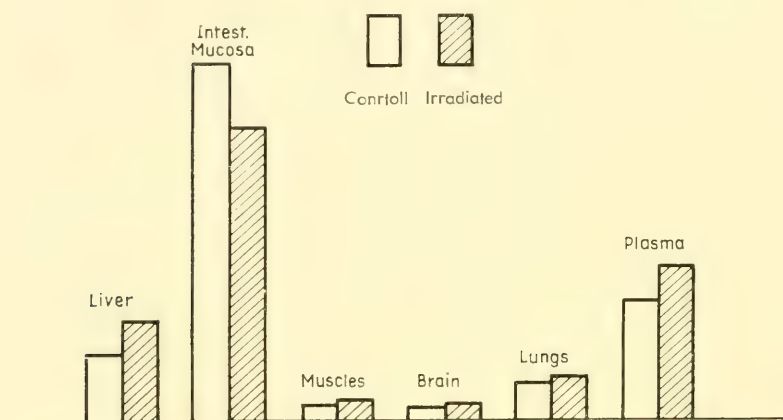


FIG. 4. Uptake of ^{14}C by 1 mgm tissue proteins of irradiated and of control mice 90 min. after intraperitoneal injection of ^{14}COO . NaCH_3 .

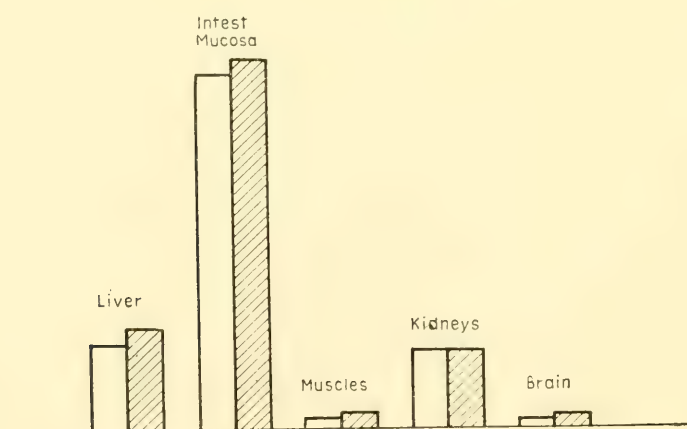


FIG. 5. Uptake of ^{14}C by 1 mgm of tissue protein of irradiated and of control mice 6 hr after irradiation and 91 min. after intraperitoneal injection of NaCH_3 ^{14}COO

diation. In this experiment no significant increase in the ^{14}C incorporation into liver proteins of the irradiated rats was observed.

When comparing the figures obtained for suckling rats with those of fully grown mice great differences are observed.

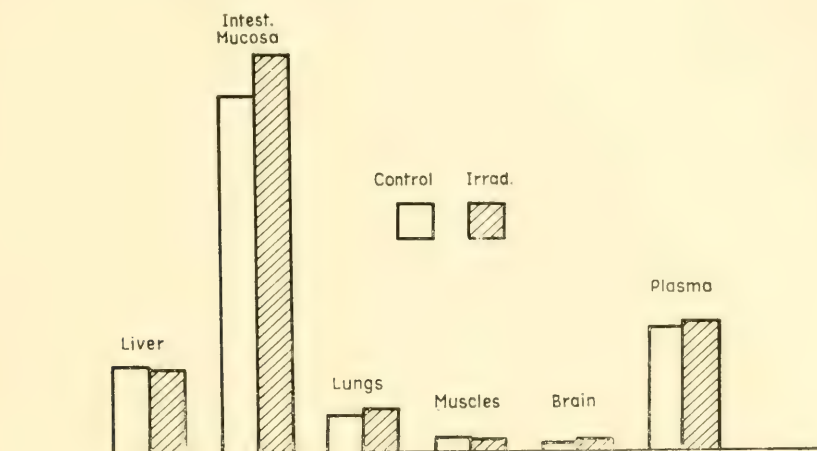


FIG. 6. Uptake of ^{14}C by 1 mgm of tissue protein of irradiated and of control mice 90 min after intraperitoneal injection of $\text{NaCH}_3 \text{ }^{14}\text{COO}$.

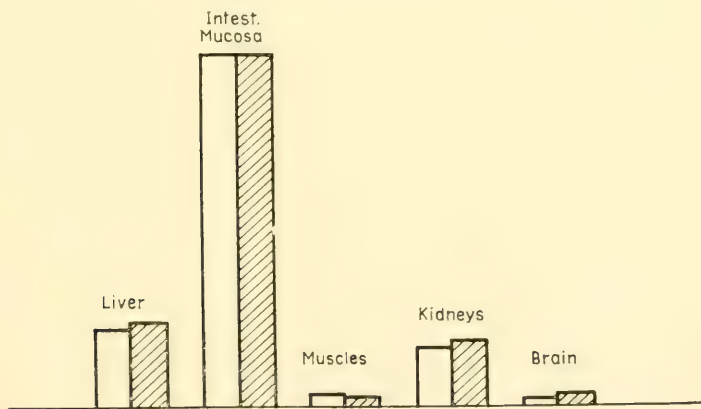


FIG. 7. Uptake of ^{14}C by 1 mgm of tissue proteins of irradiated and of control mice 24 hr after irradiation and 105 min after subcutaneous injection of $\text{NaCH}_3 \text{ }^{14}\text{COO}$.

As similar differences were observed when comparing the ^{14}C uptake by young and by adult mice in experiments carried out by FORSSBERG and one of us, the results of which will be soon published, these first mentioned differences are not mainly due to differences in the metabolic rate of rats and mice, but to a large extent to the fact that in one case

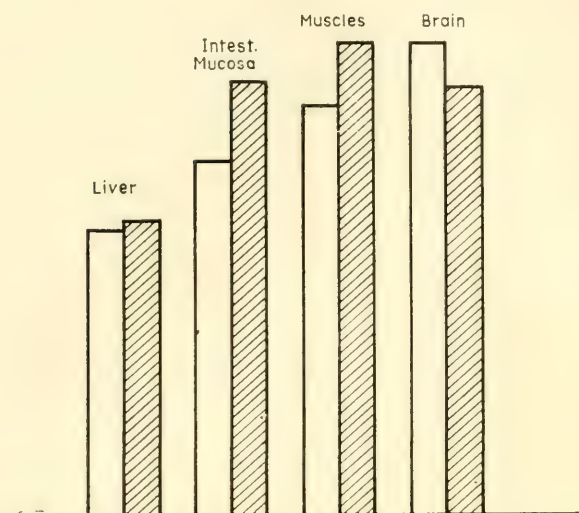


FIG. 8. Uptake of ^{14}C by 1 mgm of tissue proteins of irradiated and of control suckling rats 3 hr after intraperitoneal injection of $\text{NaCH}_3 \text{}^{14}\text{COO}$.

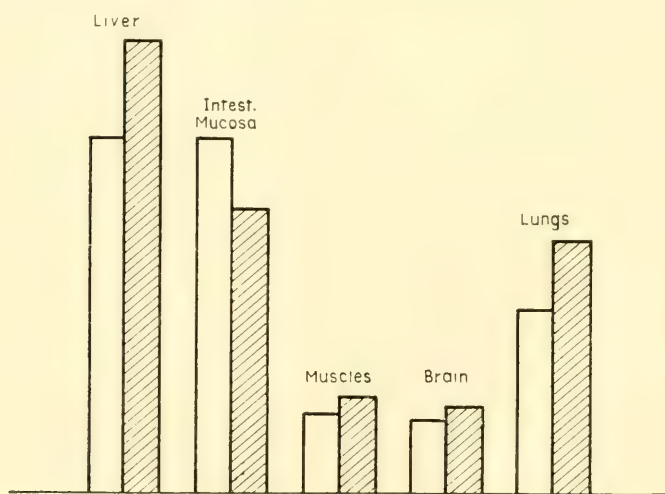


FIG. 9. Uptake of ^{14}C by 1 mgm of total fat extracted from organs of irradiated and of control mice 90 min after intraperitoneal injection of $\text{NaCH}_3 \text{}^{14}\text{COO}$.

we are dealing with rapidly growing, however, in the other case with fully grown animals.

The difference in the pattern of ^{14}C uptake by the proteins of the tissue fractions of fully grown mice and suckling rats is seen in Figs. 2—7 and 8. When plotting the figures the ^{14}C content of 1 mgm of protein secured

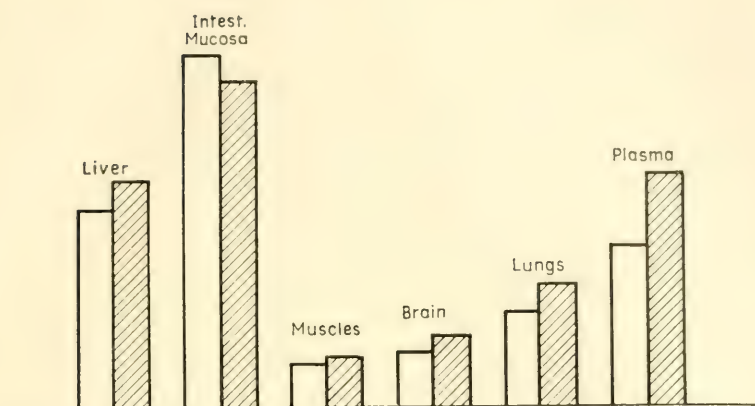


FIG. 10. Uptake of ^{14}C by 1 mgm of dry tissue of irradiated and of control mice 90 min after intraperitoneal injection of $\text{NaCH}_3\ ^{14}\text{COO}$.

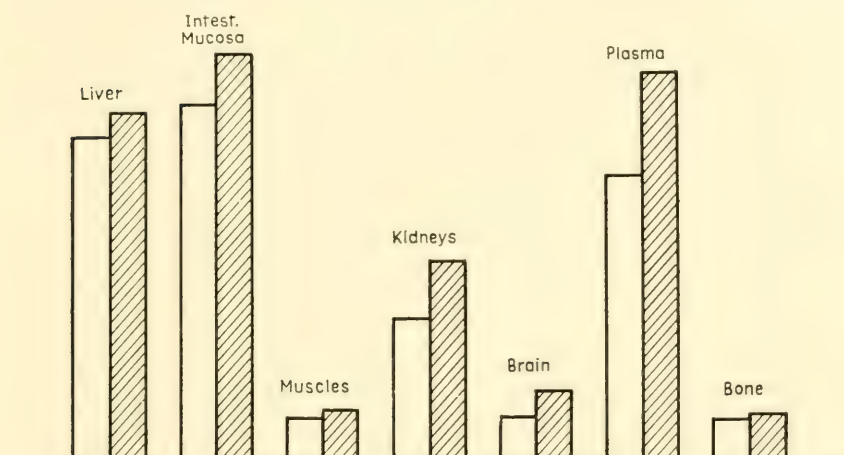


FIG. 11. Uptake of ^{14}C by 1 mgm of dry tissue of irradiated and of control mice 6 hr after intraperitoneal injection of $\text{NaCH}_3\ ^{14}\text{COO}$.

from the intestinal mucosa of control animals was taken to be 100. While as seen in Figs. 4 to 7 in the fully grown animal from all protein fractions that of the intestinal mucosa has the largest ^{14}C content, for the rapidly growing rat (cf. Fig. 8) this is no longer the case.

A comparison of ^{14}C uptake by the fatty fractions of the organs of the newly born and of outgrown animals exhibit very pronounced differences as well. While in the outgrown mouse the ^{14}C content of 1 mgm of brain fats amounts to a small percentage only of the corresponding value of the intestinal mucosa fat, in the suckling rat the ^{14}C content of 1 mgm of brain fats is higher than that of 1 mgm of intestinal mucosa fat, while in the outgrown rat in experiments taking a few hours the ^{14}C content

of 1 mgm of liver proteins is smaller than that of the liver fats, in the suckling rats the ^{14}C content of 1 mgm of liver proteins is larger than that of 1 mgm of liver fats.

The great difference in the distribution of ^{14}C between the various tissues of outgrown and rapidly growing animals is also brought out by the Figs. 8—13 in which the ^{14}C content of 1 mgm of dry tissue is stated. In these as well as in the previous figures the ^{14}C content of the intestinal mucosa is taken to be 100.

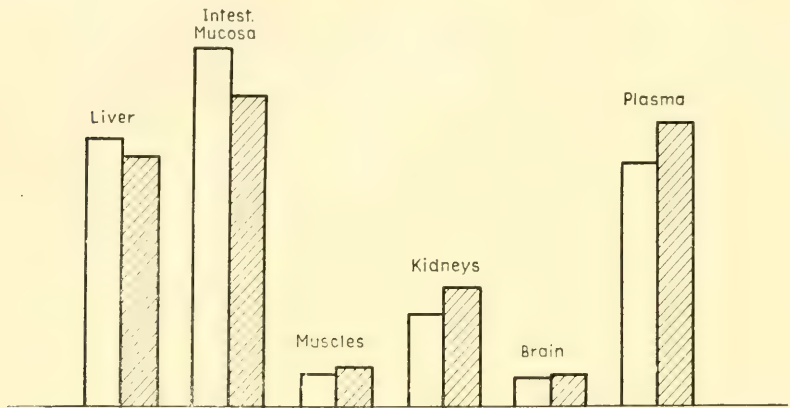


FIG. 12. Uptake of ^{14}C by 1 mgm of dry tissue of irradiated and of control mice 24 hr after irradiation and 105 min after subcutaneous injection of $\text{NaCH}_3\ ^{14}\text{COO}$

TABLE 8. — PERCENTAGE CHANGE IN THE ^{14}C UPTAKE BY TISSUE FRACTION OF FED FULLY GROWN MICE DUE TO IRRADIATION WITH 880 R.

Fraction	Liver		Intestinal mucosa		Muscles	
	% change	mean deviation	% change	mean deviation	% change	mean deviation
Dry tissue	+ 10.0	± 12.5	+ 4.6	± 6.1	+ 9.6	± 8.5
Total fats	+ 4.1	± 6.1	+ 0.9	± 5.8	+ 17.6	± 31.3
Proteins	+ 11.9	± 4.8	+ 1.9	± 4.5	- 0.2	± 3.6

Fraction	Kidney		Lungs		Brain		Plasma	
	% change	mean deviation	% change	mean deviation	% change	mean deviation	% change	mean deviation
Dry tissue	+ 17.8	± 7.8	+ 12.0	± 9.1	+ 33.6	± 10.7	+ 12.2	± 3.8
Total fats	+ 5.6	± 7.4			+ 30.5	± 5.1		
Proteins	+ 7.0	± 3.7			+ 16.0	± 6.0		

The results of the investigation of the effect of irradiation on the ^{14}C uptake by tissue fractions of fullygrown mice is seen in Table 8. This table does not contain the results obtained in our first experiment carried out with 38 mice in which the ^{14}C content of dry tissue samples alone

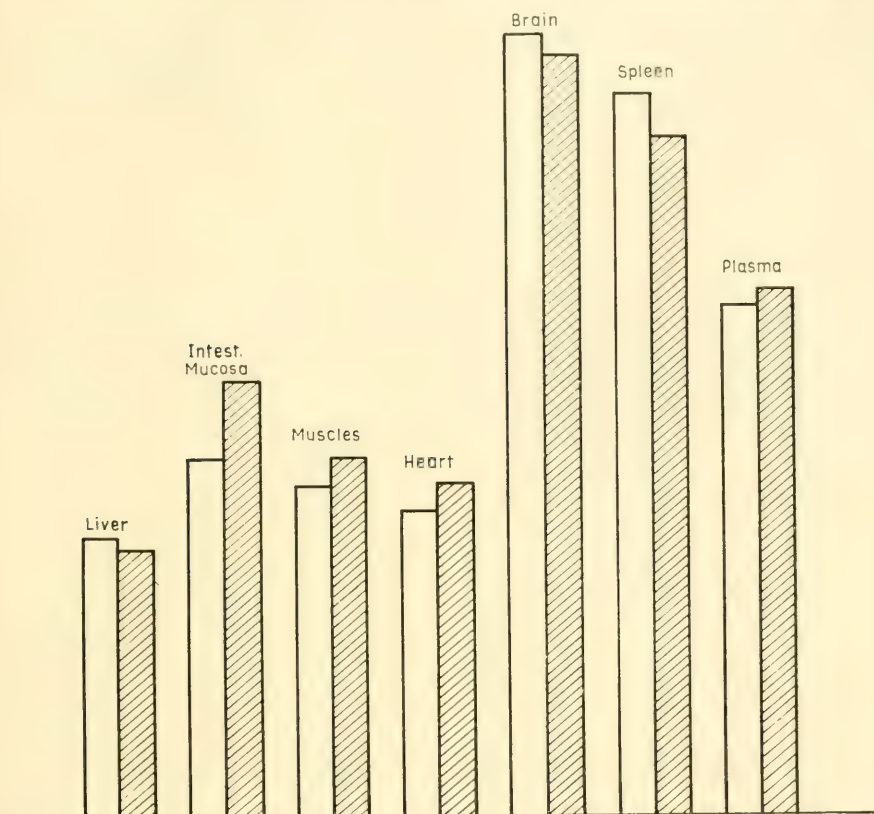


FIG. 13. Uptake of ^{14}C by 1 mgm dry tissue of irradiated and of control suckling rats 3 hr after intraperitoneal injection of NaCH_3 ^{14}COO .

was compared⁽¹⁾. In this experiment, the irradiated samples of all tissues took up appreciably more (13 to 106 per cent) ^{14}C than the controls.

As seen from Table 8 irradiation increases the mean value found for the rate of ^{14}C incorporation in all the investigated tissue fractions of fed mice except that into muscle proteins. The increase is, however, for most fractions not significant as the mean deviations are very appreciable. A suggestive increase is observed in the three brain fractions ($P = < 0.03$; < 0.005 and < 0.05) and also in the protein fraction of

⁽¹⁾ G. HEVESY, **164**, 269 (1949),

the liver ($P < 0.04$) and in the plasma fraction ($P < 0.03$). The mean value in the percentage change in the ^{14}C uptake by all 17 fractions enumerated in Table 8 works out to be 11.5, the mean deviation of this value to ± 5.3 , thus $P < 0.05$.

As much of the plasma proteins originates from the liver we have to expect to find higher ^{14}C figures in the plasma proteins of the irradiated mice, which is actually the case. A markedly increased incorporation of ^{14}C into the brain phosphatides of rats, after administration of labelled acetate was observed by ALTMAN *et al.*⁽¹⁾

Summary

Following intraperitoneal injection of acetate labelled with ^{14}C in the carboxyl group, total irradiation of 250 fed mice with 880 r administered in the course of 3 minutes leads to an increase in the mean value of ^{14}C incorporation into total tissue of liver, intestinal mucosa, muscles, kidney, lungs, brain, and plasma, into total fats of liver, muscles, kidney and brain, into the proteins of liver, kidney and brain of the mice investigated.

⁽¹⁾ K. I. ALTMAN, G. W. CASARETT, T. R. NOONAN and K. SALOMON, *Fed. Proc.* **8**, No. 1 (1949).

Originally published in *Nature*, **164**, 1007 (1949).

82. EFFECT OF MUSCULAR EXERCISE AND OF URETHANE ADMINISTRATION ON THE INCORPORATION OF CARBON-14 INTO ANIMAL TISSUE

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As a result of the observations described in a previous note⁽¹⁾, according to which irradiation with X-rays influences the pattern of incorporation of carbon-14 into animal tissue, it became particularly important to investigate the effect of muscular exercise on the incorporation of this isotope.

Five minutes after intraperitoneal administration of 4 μ curies of carboxyl labelled acetate to each individual of twenty mice, half the animals were made to swim for twenty minutes. At the end of this period these mice and an equal number of controls were decapitated. The activities were then determined of the dry tissue, of the ether-alcohol extract (fatty fraction) and the protein residue obtained by extraction with trichloroacetic acid of the organs listed in the accompanying table. Incorporation of carbon-14 is seen to be significantly decreased in almost all cases.

Similar results were obtained in 3 hr experiments where swimming was replaced by more moderate exercise, the mice being encouraged to move each time they stopped. The percentage reduction in incorporation was, however, smaller, amounting in most fractions investigated to about 5 per cent only.

In another series of experiments, 20 mgm of urethane was injected into each of thirty mice, followed by the administration of labelled acetate. The animals obtaining urethane were found after a period of 4.5 hr to have incorporated markedly more carbon-14 than did the controls. The results listed in the table, demonstrate the great sensitivity of this method, in which the incorporation of carbon-14 in tissue fractions serves as an index of particular metabolic processes. The usual methods, such as the comparison of the $Q_{CO_2}^X$ or the Q_{O_2} values of surviving tissue slices of urethane-treated mice (20 mgm per animal) and of controls do not show any significant difference⁽²⁾. On the other hand, its depressing effect on tissue metabolism is clearly brought out by the above figures.

Carbon-14 of the acetate administered can be incorporated into other carbon compounds either after oxidation to carbon dioxide or directly without oxidation. For example, acetate may contribute carbon atoms to the glucose units of glycogen by pathways other than those of oxidation and carbon dioxide fixation⁽³⁾. In both cases, increased catabolism as such produced by muscular exercise can be expected to lead to a diminished incorporation of carbon-14 into many of the tissue constituents. Following the injection of labelled acetate, apart from a brief initial period, as shown by GOULD and associates⁽⁴⁾ the specific activity of the carbon dioxide decreases very markedly with time. This decrease is interpreted as being primarily due to a dilution of radioactive carbon dioxide of the body fluid with normal carbon dioxide resulting from other metabolic processes. An increased metabolism can thus be expected to lead to an increased rate of dilution and to a correspondingly enhanced rate in the decrease of the specific activity of the labelled carbon dioxide with time, leading in turn to a decrease in the amount of carbon-14 incorporated into the tissue constituents.

EFFECT OF (a) MUSCULAR EXERCISE AND (b) URETHANE ADMINISTRATION ON THE INCORPORATION OF CARBON-14 INTO TISSUE CONSTITUENTS OF MICE. EACH MOUSE INJECTED, 26,600 COUNTS

Fraction	(a) 20 min (19.0 gm mouse)		% change	(b) 270 min (21.2 gm mouse)		% change
	Control	Swimming		Control	Urethane	
Plasma total	97.0	76.6	-21	65.0	75.4	+16
Liver total	156.5	115.2	-26	57.2	94.1	+67
Liver fatty products*	50.5	38.3	-24	15.7	29.7	+89
Liver glycogen	20.4	16.0	-22	—	—	—
Kidney total	55.5	53.1	-4	35.5	38.6	+9
Kidney fatty products	14.3	12.0	-17	6.6	7.5	+1.5
Kidney protein	15.8	16.0	+1	30.2	34.1	+13
Intestinal mucosa total	188.6	179.2	-5	143.6	117.8	+24
Intestinal mucosa fatty products	41.2	35.7	-13	24.1	36.0	+50
Intestinal mucosa protein	41.2	35.7	-13	152.4	166.6	+9
Muscle total	18.1	15.4	-15	8.2	10.8	+32
Muscle fat	8.3	10.4	+25	2.0	3.0	+50
Muscle protein	4.2	3.1	-26	4.2	5.4	+29
Brain total	23.9	20.7	-13	13.1	13.7	+5
Brain fat	5.7	5.7	0	3.6	4.0	+21
Brain protein	1.4	1.1	-21	13.7	15.2	+27

* The ether-alcohol soluble fraction were transformed into barium carbonate previous to activity measurement. Correspondingly the figures have to be multiplied by 7.7 to make them comparable with the protein-activity figures.

If the acetate carbon is incorporated into organic compounds by pathways other than those of oxidation and carbon dioxide fixation, increased catabolism will act in a similar way on the incorporation of carbon-14 into the tissue constituents. Thus labelled acetate administered may be very appreciably diluted by endogenous body acetate⁽⁵⁾ formed in the course of catabolic processes. Increased catabolism can thus be expected to promote dilution and to diminish the specific activity of the labelled acetate.

A decreased catabolism, as produced, for example, by the administration of urethane, can be expected to act in the opposite direction, slowing down the decrease in the specific activity of carbon dioxide and acetate with time and thus promoting incorporation of carbon-14 into tissue constituents.

Muscular exercise and urethane administration may influence the rate of incorporation of carbon-14 into tissue constituents by acting on other metabolic steps than those mentioned above; such an influence is indicated by the observed increase following muscular exercise in the carbon-14 content of the fatty fractions of the muscles and in some experiments also in those of the intestinal mucosa. Nevertheless, the dilution effect is presumably to a large extent responsible for the changes observed in the incorporation of carbon-14 following muscular exercise and urethane administration.

The carbon-14 content of the average total tissue is found to be about twice as large after the lapse of 20 min as after the lapse of 4.5 hr. This is mainly due to the fact that the proportion of non-metabolized acetate in the tissue is very much larger after 20 min than after 4.5 hr, and also that fatty components present, being renewed at a very rapid rate, are more active after 20 min than after 4.5 hr. The probability of carbon-14 taken up by the protein being lost again within a few hours is small, and correspondingly the average tissue protein activity after the lapse of 20 min is about one-third only of the value observed after the lapse of 4.5 hr.

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COMMENT ON PAPERS 79—82

AFTER observing a depressed ^{32}P incorporation into DNA of the irradiated rat, we investigated the effect of exposure on ^{14}C incorporation into the purines of DNA of various organs. The only ^{14}C labelled material at our disposal being at that date sodium acetate, we studied acetate carboxyl ^{14}C incorporation into the purines of DNA of several organs of growing control irradiated mice. A depression of about 50 per cent was observed (paper 79), thus the same as found when studying the effect of irradiation on ^{32}P incorporation. Incorporation of ^{14}C into proteins was not found to be depressed, a slightly increased incorporation was observed. Somewhat increased ^{14}C incorporation into the proteins following irradiation of the animal was also found by BUTLER *et al.* (1956) and in some protein fractions by RICHMOND *et al.* (1957). The total tissue of the organs of exposed mice investigated had a higher content of ^{14}C than the organs of controls (paper 81). A similar result was found after administration of urethane. Muscular exercise (thus enhanced metabolism) led to a decreased incorporation of ^{14}C into the tissues (paper 82). In recent years increased isotope incorporation into constituents of exposed animals was observed in numerous cases (BACQ and ALEXANDER, 1955). A blockage of the incorporation of ^{14}C into DNA will leave more ^{14}C available for entering other tissue constituents. This may explain, at least to some extent, the increased rate of formation of some ^{14}C labelled compounds in the organism exposed to irradiation. The latter may, however, also be due to a cell lesion followed by an activation of enzymes. HAGEN (1958) for example, recently found an increase in the cathepsin activity of the thymus and spleen of the irradiated mouse, and it is probable that this increase is due to a release of the enzyme by the damaged cells.

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83. EFFECT OF IRRADIATION BY X-RAYS ON THE EXHALATION OF CARBON DIOXIDE BY THE MOUSE

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It has been shown previously that the rate of formation of deoxyribonucleic acid is depressed by a sublethal dose of X-rays. Both the incorporation of phosphorus-32⁽¹⁻⁵⁾ and that of carbon-14^(6, 7) was found to be reduced about 50 per cent. The rate of incorporation of carbon-14 into the purines of ribonucleic acid is reduced as well⁽⁷⁾, though to a smaller extent than its incorporation into the purines of deoxyribonucleic acid. These blocking effects are presumably partly due to inactivation of enzymes involved in the synthesis of the deoxyribonucleic acid and ribonucleic acid compounds. One or a few days after irradiation, the incorporation of carbon-14 into purines of deoxyribonucleic acid has almost returned to the rate for the non-irradiated controls. This suggests that a partial reactivation has occurred. The early metabolic changes produced by irradiation are not confined to interference with nucleic acid synthesis. We find that irradiation interferes with glucose metabolism as well.

Fully grown mice which had been fed *ad libitum* were placed in perforated metal boxes which prevented gross movement. In each experiment four such boxes were symmetrically placed in a metal cylinder containing carbon dioxide-free air and kept at 27°. Exhaled carbon dioxide was swept from the brass cylinder by carbon dioxide-free air at constant velocity and collected in a series of three wash-bottles containing barium hydroxide. At intervals of 10 min the air stream was switched over to another aggregate of wash-bottles. The carbon dioxide was collected for one hour.

Glucose (60 μ g having an activity of 0.03 μ C.) labelled in all its carbon atoms was injected subcutaneously into each mouse immediately after irradiation. Collection of carbon dioxide started 8 min after injection. The increase in weight of barium carbonate with increasing time shown in the graph has no significance, as it is a result of the fact that the content of exhaled carbon dioxide in the metal cylinder increases with time. We wished only to determine the ratio of carbon dioxide exhaled by irradiated and control mice, not the total amount exhaled.

We found that the non-fasting irradiated mice exhale only some 80 per cent of the amount of carbon dioxide exhaled by control mice. This result does not necessarily prove an impaired catabolism of body glucose. Since only about one-half of the exhaled carbon dioxide originates from catabolism of glucose⁽⁸⁾, the other half having its origin in catabolism of fatty acids and other body constituents, it may be that the catabolism

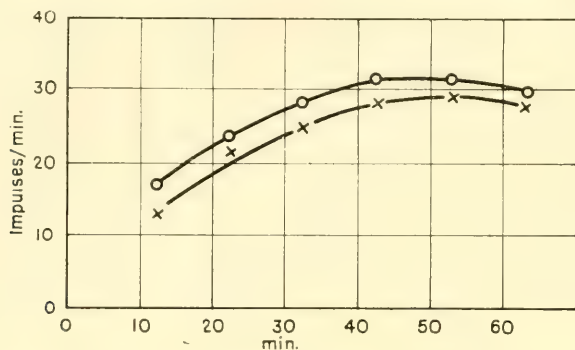


FIG. 1. Effect of irradiation with 2000 r. on the production of exhaled carbon-14 dioxide after injection of labelled glucose to non-fasting mice. O, control; X, irradiated.

of the last-mentioned compounds and not that of glucose is responsible for the reduced exhalation of carbon dioxide by the irradiated mouse. The fact, however, that if labelled glucose is administered the irradiated mice exhale a smaller amount of carbon-14 labelled carbon dioxide than the controls, proves unambiguously that irradiation depresses the glucose catabolism of irradiated mice. The graph indicates that the depression is already apparent after the lapse of 12 min and may possibly be still more pronounced at an earlier time.

It is of interest to note that LOURAU and LARTIGUE observed that addition of glucose to the diet of the guinea pig increases its radiosensitivity⁽⁹⁾, that irradiation with 500 r. produces hyperglycaemia in the irradiated animal⁽¹⁰⁾ and, quite recently, that 12–15 days after irradiation glycogen formation in the liver is reduced 1–2 hr after feeding glucose⁽¹¹⁾.

The reduced catabolism of glucose in the irradiated mouse is reflected in an increase of forty to one hundred per cent in the carbon-14 content of liver fats.

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84. EFFECT OF X-RAYS AND HORMONES ON RESORPTION RATE OF INJECTED $\text{NaH}^{14}\text{CO}_3$

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THIS paper reports observations on the effect of X-irradiation on the rate of exhalation of $^{14}\text{CO}_2$ by mice, injected with ^{14}C -labelled sodium bicarbonate. The possibility that X-ray effects were mediated through hormonal action led us to investigation of the effects of ACTH as well as of adrenaline, administered alone, or in combination with irradiation.

The mean life-time of the bulk of the circulating bicarbonate ions in the body of the mouse being some minutes only, any change in the rate of resorption will be reflected in a corresponding change in the amount of $^{14}\text{CO}_2$ exhaled within a few minutes or even seconds after the injection. Following the rate of exhalation of $^{14}\text{CO}_2$ is thus a sensitive and convenient measure of a changed resorption rate of the intraperitoneally, subcutaneously or intramuscularly injected labelled bicarbonate. Such investigations may also supply information on the factors governing the resorption process.

MATERIAL AND METHODS

White mice weighing 15–25 gm and fed on standard vitaminized biscuits were injected intraperitoneally, subcutaneously or intravenously into the tail vein with 0.1 ml of saline containing 10–50 mgm of labelled sodium bicarbonate having an activity of 0.5–0.3 μc . A precision "Agla" syringe was used. Each of a group of four mice was placed in a small perforated aluminium box. These boxes were then inserted into a metal cylinder of 2.5-liter volume through which a CO_2 -free air stream of 150 ml/min passed. The exhalatory CO_2 was led through three centrifuge glasses containing a $\text{Ba}(\text{OH})_2$ solution. Every 10 minutes the air stream was directed into another set of centrifuge bottles. The precipitated BaCO_3 was collected, washed and dried. The weight and radioactivity of the precipitate were determined.

When following the exhalation of $^{14}\text{CO}_2$ from the start of the injection we used another arrangement, placing each mouse into a brass tube of 4 cm diameter and 15 cm length. The mouse was injected after having been placed into the brass tube through which air passed immediately after the injection which took 15 seconds. The air stream carrying the exhalatory CO_2 was diverted every 2 minutes into another set of centrifuge bottles. Contrary to the first described procedure,

we added in these experiments to each centrifuge bottle NaHCO_3 carrier before collecting the precipitated BaCO_3 for assay of radioactivity.

For determination of the total amount of exhaled CO_2 per each 2-minute period with this latter procedure no carrier was added. The precipitate was spun down and the supernatant solution was titrated to $\text{pH} \sim 7$, spun down and poured off. After two washings with ice cold CO_2 free distilled water alcohol the precipitate was dissolved in HCl and backtitrated with NaOH .

In the X-ray experiments every second group of mice was, previous to injection, exposed to 2000 r of 155 kv X-rays filtered by 1 mm Al, dose rate 140 r/min. in the experiments where CO_2 was collected in 10-minute intervals and 1350 r/min. in those series collecting CO_2 in 2-minute intervals.

The data relevant to the hormone administration are given in connection with the description of those experiments.

RESULTS

Effect of X-Rays

In a set of eight experiments the exhalation of the total amount of CO_2 and of $^{14}\text{CO}_2$ from groups of each four irradiated and four control mice was measured using the first arrangement described above. It appeared that the irradiation slightly decreased the amount of CO_2 exhaled ($12 \pm 2.7\%$) as found by us previously. (1) The amount of $^{14}\text{CO}_2$ (milligrams $\text{CO}_2 \times$ relative specific activity) exhaled by the exposed mice, however, was significantly greater ($22.8 \pm 3.4\%$) than that given off by the controls. The collection of CO_2 was started between 15 and 30 minutes after the injection of the $\text{NaH}^{14}\text{CO}_3$, which followed immediately upon the irradiation and was continued in 10-minute intervals for about 1 hour. No conspicuous changes in the percentage figures were found during that time.

Essentially, similar results were found when $\text{NaH}^{14}\text{CO}_3$ was injected 20 hours after the irradiation giving $18.7 \pm 2.4\%$ less CO_2 and $19.9 \pm 3.9\%$ more $^{14}\text{CO}_2$ on an average. It appears, then, that these X-ray effects persist for at least 1 day. It is possible, however, that this late effect is of another character, being the result of a progressive irradiation lesion.

The enhanced exhalation of $^{14}\text{CO}_2$ might be expected to run parallel with a smaller amount of residual ^{14}C in the irradiated animals. Analyses of control and irradiated animals killed 40 minutes after injection showed, however, that the activity of the homogenized and dried tissue did not vary appreciably. Out of the injected 106,000 counts, 5060, i. e. 4.8%, were found to be present in the controls and 4850, i. e. 4.6%, in the exposed mice. The enhanced exhalation of $^{14}\text{CO}_2$ by exposed animals could thus not be due to an increased retention of ^{14}C by the controls.

The only explanation left seemed to be that irradiated mice exhale $^{14}\text{CO}_2$ at a markedly decreased rate for a very short period immediately

after the injection of the bicarbonate, leaving these animals with an increased pool of ^{14}C some 10–60 minutes afterwards. This conclusion induced us to carry out corresponding experiments in which collection of $^{14}\text{CO}_2$ was started immediately after the injection, making use of the second arrangement described above.

The results of these experiments are plotted in Figure 1. In the first 4 minutes of the experiment during which a very appreciable percentage

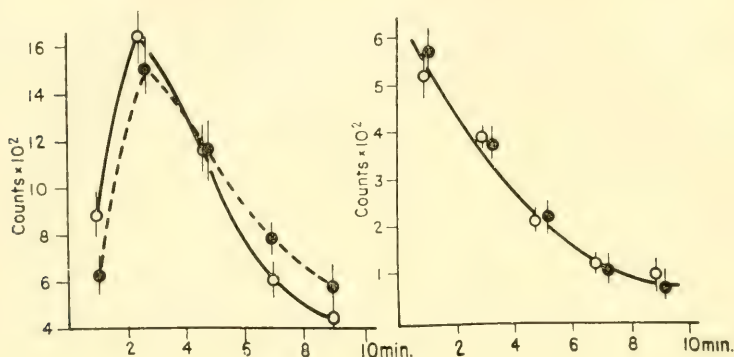


FIG. 1. Exhalation of $^{14}\text{CO}_2$ of x-rayed (●) and control (○) mice after intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$. Values for the determinations of $^{14}\text{CO}_2$ in the interval 0–2 minutes are plotted at 1 minute, etc.

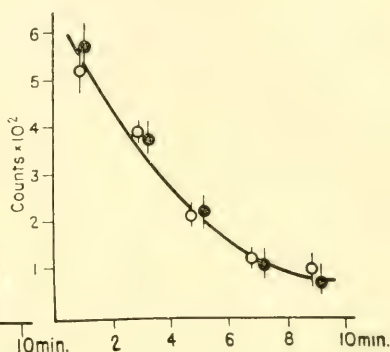


FIG. 2. Exhalation of $^{14}\text{CO}_2$ of x-rayed (●) and control (○) mice after intravenous injection of $\text{NaH}^{14}\text{CO}_3$. Less activity than in the intraperitoneal experiments was injected.

of the injected $^{14}\text{CO}_2$ is exhaled, the controls gave off more $^{14}\text{CO}_2$ (45%) than the irradiated animals (29%), and more $^{14}\text{CO}_2$ was retained in the exposed animals which gave off more $^{14}\text{CO}_2$ in the later stage of the experiment. Between 4 and 10 minutes 46% was given off by the irradiated animals against 34% by the controls. The aggregate amount of $^{14}\text{CO}_2$ exhaled during the first 10 minutes by the two groups of mice is by chance the same as seen in Table 1. The results are remarkably reproducible.

Evidently it would be expected that the activity expired by the irradiated mice during the first minute would be decreased even more from that of the control mice. In a few trials of this hypothesis the activity expired during the 1st minute, including the 6 seconds taken for the injection in these experiments, was found to be only 43% of that of the controls. This indicates that the X-ray effect on the process of resorption of the bicarbonate and the intrusion of CO_2 into the alveolar space is very marked.

In the later part of the experiment the amount of $^{14}\text{CO}_2$ expired, decreases more and more with increasing time. After the lapse of 30 minutes the amount exhaled in the course of 10 minutes made up only about 1% of the injected quantity. It is, therefore, difficult to judge whether the 20—25% higher output of activity from irradiated mice some 30 minutes after the injection of the bicarbonate may be explained solely by an initial lag very shortly after injection. As, however, irradiation influences the incorporation of ^{14}C into different body constituents and as incorporation and release of ^{14}C are intimately connected, a preferential release of ^{14}C from the compounds of the irradiated mice may contribute to the increased exhalation in the later part of the experiment.

The slower exhalation of $^{14}\text{CO}_2$ by the exposed animals shortly after injection of the labelled bicarbonate could be due to a depressed resorption and/or a circulation disturbance. To investigate the latter possibility we injected labelled bicarbonate into the tail vein of both control and irradiated mice and collected the exhalatory CO_2 in 2-minute intervals starting immediately after injection. No conspicuous differences could be noticed in the amount of $^{14}\text{CO}_2$ exhaled as shown in Figure 2. The depressed exhalation of $^{14}\text{CO}_2$ by the exposed mouse is probably caused by a decreased resorption rate of the injected bicarbonate and not by circulation disturbances.

We investigated also the effect of irradiation on the exhalation of $^{14}\text{CO}_2$ following a subcutaneous injection. The results obtained in six experiments varied much more than those observed after intraperitoneal injection and do not permit definite conclusions on the effect of irradiation on the rate of resorption. The mean value of the aggregate amount exhaled in the course of 10 minutes was 57.7% for the controls and 45.5% for the X-rayed animals compared with 65.8 and 65.3% after intraperitoneal injection. The amount exhaled in the first 4 minutes varied between 9.6 and 34.6%.

TABLE 1. — PERCENTAGE OF ACTIVITY
ADMINISTERED EXHALED IN THE
COURSE OF THE FIRST 10 MIN.

Exper.	Control	X-Rayed
1	79.5	79.4
2	79.9	79.9
3	50.5	50.4
4	57.9	58.1
5	61.1	58.7
Mean	65.8	65.3

The irradiated and control mice had in each experiment within 5% identical weights, which, however, differed from experiment to experiment.

Resorption is presumably mediated through hormonal action. This line of thought induced us to investigate the effect of the administration of ACTH and adrenaline on the rate of exhalation of $^{14}\text{CO}_2$ following intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$. In some of these experiments the administration of the hormones was combined with irradiation.

Effect of ACTH

In two sets of experiments the mice were injected subcutaneously on the 2 days preceding the experiment with 0.04 i.u. of ACTH (Acton Prolongatum) to each mouse, and 1 hour before the injection of $\text{NaH}^{14}\text{CO}_3$ with five times that amount. In a third set of experiments 0.008 i.u. of ACTH was administered for 3 days preceding the experiment and besides 0.04 i.u. 20 minutes before the injection of $\text{NaH}^{14}\text{CO}_3$. The CO_3 was collected in 2-minute periods as described above. The three sets of experiments did not differ significantly from each other; possibly the effect of ACTH was slightly greater when the main dose was administered 20 minutes before the injection of the $\text{NaH}^{14}\text{CO}_3$.

As seen in table 2A (*group 1*) administration of ACTH accelerates the exhalation of $^{14}\text{CO}_2$ markedly. On an average 25% more $^{14}\text{CO}_2$ is exhaled in the first 2 minutes; the difference is statistically significant. The total output of CO_2 is decreased (*group 2*). As expected, less activity is expelled during the following minutes. That process was not followed up any longer than necessary to demonstrate the principle. In the following groups of experiments only the interval 0—2 minutes was studied because the differences are most obvious immediately after the injection of the isotope.

As shown in figure 1, irradiation alone caused an immediate depression in the exhalation of $^{14}\text{CO}_2$, while ACTH administration increased it. When irradiating the mice after an ACTH administration (the last injection 20 min before the irradiation) it appears that this combined treatment brings about an increase in the output of $^{14}\text{CO}_2$ amounting to +32.3% over that obtained from animals being only irradiated (table 2A, *group 3*). Alternatively, when comparing the effect of the combined treatment with untreated controls no difference in the output of $^{14}\text{CO}_2$ could be noticed. Thus, an ACTH treatment previous to the irradiation annuls the X-ray effect.

Effect of Adrenaline

The experiments with adrenaline were mainly performed in a similar way. Adrenaline was administered only once, 5—15 minutes before the injection of the labelled bicarbonate. The dose was 2 μgm adrenaline per mouse, given subcutaneously (Table 2B). Evidently adrenaline acts in

a manner which is mainly contrary to that of ACTH. The output of $^{14}\text{CO}_2$ (Table 2B, group 1) is markedly decreased in association with a possible slight increase in the total amount of CO_2 (group 2). The latter figure is, however, not statistically significant. The combination of adrenaline and irradiation, administering adrenaline 5–15 minutes before the irradiation, gave a rather unexpected result. Instead of an additional decrease in the output of $^{14}\text{CO}_2$ over that resulting from the application of one of these two agents alone, an increased output was obtained (group 3). Determinations of the total amount of exhaled CO_2 in this combined treatment gave a quite normal figure (group 4).

Discussion

The CO_2 of the circulating bicarbonate is in rapid exchange equilibrium with the CO_2 of the alveolar air. Consequently, after an initial stage following injection of labelled bicarbonate, the specific activities of the blood CO_2 and that of the expired air become identical.

Apart from exhalation, which represents its main route, numerous competitive routes are open to the $^{14}\text{CO}_2$ in the animal body. $^{14}\text{CO}_2$ finds its way into almost any organic body constituent. The injected $\text{NaH}^{14}\text{CO}_3$ gives off its $^{14}\text{CO}_2$ to the alveolar air so rapidly, however, that only a small amount is incorporated into the tissue constituents. We found 40 minutes after the administration of labelled bicarbonate the body of the mouse to contain 4.8% of the injected ^{14}C . SKIPPER *et al.*^(2, 3) record the presence after the lapse of 25 hours of 1.37% and after 12 weeks of 0.12% of the total dose injected. The share of the muscles in the total body $^{14}\text{CO}_2$ amounts after 25 hours to 41% and after 12 weeks to 27%. For the skeleton, the figures given by SKIPPER were: 18% is present after 25 hours and 23% after 12 weeks. The skeleton gives off its $^{14}\text{CO}_2$ at a lower rate than the muscle or any other organ with the exception of the skin and hair. ARMSTRONG *et al.*⁽⁴⁾ found half of the $^{14}\text{CO}_2$ incorporated into the skeleton of the rat after the lapse of 8 days still to be present after 1 month.

We found the rate of exhalation of $^{14}\text{CO}_2$ following intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$ to be markedly depressed after exposure of the mouse to 2000 r while that of total CO_2 was depressed only slightly. After intravenous injection of $\text{NaH}^{14}\text{CO}_3$ the amount of $^{14}\text{CO}_2$ expired by the irradiated mouse is not depressed to any marked extent. It is thus the rate of resorption of sodium bicarbonate which is diminished through exposure. Due to the extreme rapidity of the exhalation process, the blocking effect of the irradiation on the rate of resorption of H^{14}CO_3 is only observable very briefly after the injection of the labelled bicarbonate. Thus, these X-ray effects may not be detected if the collection of the expired CO_2 is not started immediately after injection.

TABLE 2. — EFFECT OF ACTH AND OF ADRENALINE, ALONE AND IN COMBINATION WITH X-IRRADIATION, ON THE EXHALATION OF TOTAL CO₂ AND OF ¹⁴CO₂ AFTER INTRAPERITONEAL INJECTION OF NAH¹⁴CO₃

Group	Exper. Treatment	Collection of CO ₂ After In- jection, min.	Change in Ex- halation Caused by Treatment, %	Significance Test for the Diff. Between Control and Treated Animals		
				<i>n</i>	<i>t</i>	<i>P</i>
A) ACTH						
1	Comparing effect of ACTH with untreat- ed animals (given saline). Output of ¹⁴ CO ₂ measured	0—2 2—4 4—6	+ 25.7 + 6.2 — 6.3	28	3.18	0.005
2	Treatment as in <i>group</i> <i>I</i> . Total output of CO ₂ measured	0—2	— 18.3	14	2.33	0.05
3	Comparing ACTH + + irradiation with irradiation alone. Output of ¹⁴ CO ₂ measured	0—2	+ 32.3	14	2.86	< 0.02
B) Adrenaline						
1	Comparing effect of adrenaline with un- treated animals (given saline). Out- put of ¹⁴ CO ₂ mea- sured	0—2 2—4 4—6	— 30.5 + 4.2 + 6.3	12	5.86	< 0.001
2	Treatment as in <i>group</i> <i>I</i> . Total output of CO ₂ measured	0—2	+ 8.2	30	1.07	0.3
3	Comparing adrenaline + irradiation with controls. Output of ¹⁴ CO ₂ measured	0—2	+ 29.2	12	2.98	< 0.02
4	Treatment as in <i>group</i> <i>3</i> . Total output of CO ₂ measured	0—2	+ 2.3	16	0.445	0.6—0.7

SKIPPER *et al.* measured the amount of ¹⁴CO₂ expired by the mouse after intraperitoneal injection of labelled bicarbonate. They found 66% of the injected ¹⁴CO₂ to be exhaled within the first 10 minutes, 92.8% within 1 hour and 96.1 within 24 hours. We found 1—2% to be expired in the course of the 1st minute which includes the time of the injection taking 6 seconds, the time of resorption, that of reaching the alveolar space and of exhaling. Ten to sixteen % was found after 2 minutes and 65—66% after 10 minutes in agreement with SKIPPER's figure.

The resorption of bicarbonate is presumably under hormonal control. This is supported by our finding that a subcutaneous administration respectively of ACTH or adrenaline, markedly changes the rate of resorption of intraperitoneally administered sodium bicarbonate. To some extent also, these hormones influence the exhalation of total CO_2 in the mice.

As will be found from the Tables 2A and B, the total amount of CO_2 exhaled in the first 2 minutes varies slightly according to the treatment in each case. It may be argued, then, that these variations in the total amount of CO_2 would influence the corresponding determinations of $^{14}\text{CO}_2$. The amount of carrier (Na_2CO_3) added in the latter experiments was, however, equivalent to a precipitate of 200 mgm BaCO_3 . The output of CO_2 in the untreated control group corresponds to about 26 mgm BaCO_3 during the first 2 minutes. The same figure in the group administered ACTH was lower by 18.3% (Table 2A, group 2) or about 21 mgm. Thus, the differences in the total amounts of BaCO_3 received is negligible. The values in the table for 'percentage change in the exhalation caused by treatment' apply, therefore, as well to determinations of the total amount of exhaled $^{14}\text{CO}_2$ as to specific activity determinations.

It appears that the administration of ACTH causes a slight decrease in the total output of CO_2 . In this respect, ACTH acts like irradiation. The rate of resorption of the injected H^{14}CO_3 is, however, markedly increased and in this respect ACTH acts oppositely to irradiation. It may be considered whether the diminished resorption rate observed in the exposed mouse is due to a blocking effect of the irradiation on ACTH formation. The biological half-life of the injected ACTH is about 5 minutes and the half-life of the ACTH secreted into the circulation may have a similar value. (5) Blocking of ACTH formation through irradiation would manifest itself correspondingly soon. It seems to be in accordance with this hypothesis that an injection of ACTH shortly before irradiation neutralizes the effect of X-rays, as visualized in Table 2A, group 3.

We investigated also the effect of adrenaline on the rate of resorption of intraperitoneally injected $\text{NaH}^{14}\text{CO}_3$. An early depression of the amount of $^{14}\text{CO}_2$ exhaled by the adrenaline injected mice was observed (Table 2B, group 1), in accordance with the finding in the X-ray experiments (cf. Fig. 1).

Administration of adrenaline may produce vasoconstriction which depresses the rate of resorption. This was found to be the case when the rate of disappearance of intramuscularly injected ^{24}Na from the place of injection was measured in human subjects to whom adrenaline had been administered. (6) On the other hand, administration of adrenaline stimulates the formations of ACTH, a substance which favours resorption. Presumably, however, the direct effect of the administered adrenaline may be expected to prevail in our experiment. The decrease in the rate

of exhalation of $^{14}\text{CO}_2$ found by us seems, therefore, to be consistent with the fact that adrenaline depresses the resorption rate of the injected $\text{NaH}^{14}\text{CO}_3$.

It is established that O_2 consumption and CO_2 formation are increased after adrenaline injection of such amounts as were used in our experiments. (7) One may expect, therefore, an increase in the total output of CO_2 in animals given adrenaline. We found only a very slight increase (cf. Table 2B, group 2). Considering that irradiation causes a decrease in the exhalation of CO_2 one may assume that adrenaline influences the exhalation of CO_2 in an opposite way to irradiation. In the combined application of adrenaline and X-rays the total output of CO_2 is close to the control value (Table 2B, group 4). This can be understood as a counterbalance of the adrenaline effect by the subsequent irradiation.

The working hypothesis that the irradiation effect on the exhalation of $^{14}\text{CO}_2$ is mediated through an interaction of adrenaline, implies that x-rays cause a release of adrenaline in the circulation. However, this is not the case. H. EULER kindly determined the adrenaline content of urine of rats irradiated with 1000 r and of controls. The adrenaline excretion was found to be 0.0040–0.0049 $\mu\text{gm/hr}$ in controls against 0.0031–0.0037 $\mu\text{gm/hr}$ X-rayed. Considering that irradiation and administration of adrenaline both influence the resorption of $\text{NaH}^{14}\text{CO}_3$ in a similar way it is also spectacular that an additional irradiation following the adrenaline injection completely reverses the decrease in the resorption rate, causing an increase above the normal value (Table 2B, group 3). This point needs further clarification, in particular by administering irradiation before the adrenaline treatment.

The studies of the effect of administering hormones on the exhalation of $^{14}\text{CO}_2$ from intraperitoneally injected $\text{NaH}^{14}\text{CO}_3$ were warranted on the assumption that the corresponding X-rays effects were mediated via hormonal actions. The results obtained in our experiments do not seem to refute such an assumption with regard to ACTH, although definite proof is difficult to achieve. Apart from the possible mediation of hormones in the X-ray effect, our experiments have shown that ACTH and adrenaline *per se* influence the resorption and exhalation of $^{14}\text{CO}_2$ from administered bicarbonate.

Summary

$^{14}\text{CO}_2$ exhalation following intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$ is markedly decreased after irradiation of mice with 2000 r X-rays. This is due to a depressed resorption rate of the sodium bicarbonate. Because of the rapid exhalation of $^{14}\text{CO}_2$ this effect appears during the first minutes after injection of the isotope, and reverses 5–6 minutes afterwards to give an increased output of $^{14}\text{CO}_2$.

The effect of subcutaneous injection of ACTH and adrenaline on the same process was investigated according to the hypothesis that the X-ray effects may be

mediated through hormonal action. ACTH increased the $^{14}\text{CO}_2$ exhalation *per se* and an ACTH treatment before irradiation annihilated the effect of the latter. It is not out of the question, therefore, that the X-ray effect is due to a blocking of ACTH formation. Adrenaline decreased the output of $^{14}\text{CO}_2$ similarly to irradiation, but irradiation was not found to release adrenaline in the body. A combined treatment, giving adrenaline before irradiation, caused a higher output of $^{14}\text{CO}_2$ than from control mice. These facts are not compatible with the hypothesis that the X-ray effect is mediated through adrenaline action.

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85. NOTE ON THE EFFECT OF X-RAYS AND HORMONES ON THE RESORPTION RATE OF INJECTED $\text{NaH}^{14}\text{CO}_3$

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In a previous communication (FORSSBERG and HEVESY, 1955), evidence was presented for an effect of exposure to X-rays on the rate of resorption of injected ^{14}C -labelled sodium bicarbonate as indicated by the rate of exhalation of $^{14}\text{CO}_2$. The circulating bicarbonate in the body of the mouse has a mean life-time of a few minutes only and consequently any change in the resorption rate will be indicated by a corresponding change in the amount of exhaled $^{14}\text{CO}_2$ within the first minutes after the injection of the labelled bicarbonate.

The percentage $^{14}\text{CO}_2$ exhaled by irradiated mice in the first 4 minutes following injection was found to be 29% of the amount administered which compares with 45% exhaled by the non-irradiated controls. In a later stage of the experiment as in the 6—10 min time interval, the amount of $^{14}\text{CO}_2$ exhaled by the exposed animals was found to be larger than that exhaled by the controls as the early preferential exhalation by the controls led to an accumulation of $^{14}\text{CO}_2$ by the exposed mice.

An X-ray effect did not manifest itself when administering the bicarbonate by intravenous injection. As the effect observed may have been mediated through hormonal action on the resorption process, we investigated the effect of both adrenaline and ACTH-administration on $^{14}\text{CO}_2$ exhalation. Subcutaneous injection of adrenaline was found in our previous investigation to decrease the rate of exhalation of $^{14}\text{CO}_2$ in a similar way as did irradiation, whereas injection of ACTH increased the immediate output of $^{14}\text{CO}_2$. Experiments in which exposure to radiation and hormonal treatment were combined brought out that an ACTH injection given shortly before irradiation annihilates the effect of the latter. The last mentioned observation can possibly be explained by an immediate depressive effect of X-irradiation on the resorption of $\text{NaH}^{14}\text{CO}_3$ due to a momentary blockage of the release of ACTH. The combined effect of an adrenaline injection and irradiation had the unexpected effect that the initial output of $^{14}\text{CO}_2$ was much higher than that from control mice although each agent alone caused a depressed $^{14}\text{CO}_2$ exhalation. The mechanism inherent in this synergistic action is obscure.

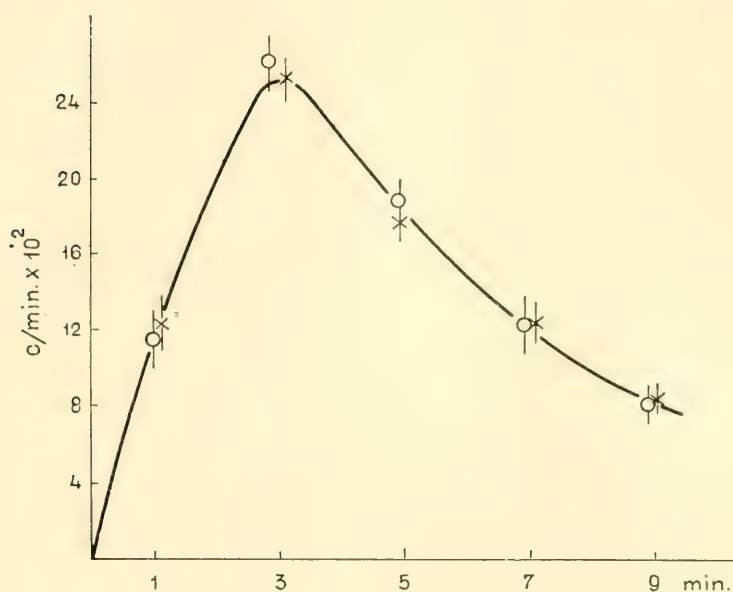


FIG. 1.

When following up these experiments half a year later — the experimental arrangement being the same as in the foregoing paper — we found irradiation with 2000 r not to influence the exhalation of $^{14}\text{CO}_2$ which follows injection of bicarbonate (Fig. 1). Concomitantly a shift in the reaction of the animals against adrenaline had occurred. In the previous investigation 2 γ adrenaline, given subcutaneously 15 min before the injection of the labelled bicarbonate, was found to decrease the $^{14}\text{CO}_2$ -output by about 30% in contrast to an increased output of 24% found in the present investigation (Table 1).

TABLE 1

Hormone	Amount of subcutaneously injected hormone	Change in initial exhalation of $^{14}\text{CO}_2$ caused by hormone injection %	Significance test for the diff. between control and treated animals		
			n	t	P
Adrenaline	2 γ , 15 min. before $\text{NaH}^{14}\text{CO}_3$ (¹)...	+ 23.9	14	2.09	0.05
„	10 γ , 15 min. before $\text{NaH}^{14}\text{CO}_3$	— 25.9	19	1.93	0.05
ACTH	On the two days prior to experiment 0.008 I. U. On day of experiment 0.04 I. U. 10 min. before $\text{NaH}^{14}\text{CO}_3$ (¹)	+ 24.8	52	2.08	0.05

(¹) Similar to the procedure in the previous investigation.

An adrenaline dose increased to 10 γ resulted, however, in a reaction which was qualitatively and quantitatively similar to that observed in our earlier investigation after administration of 2 γ . Likewise, after increasing the X-ray dose to 4000—5000 r a similar initial depression of the resorption rate was observed as was found after 2000 r in the first investigation. The influence of the ACTH-treatment was quantitatively the same on both the two occasions.

No definite opinion can be expressed concerning the reason for the change in the sensitivity of mice to exposure and to adrenaline administration; whether it is based on seasonal variations (the first investigation was performed in winter—spring, this second one in the autumn) or is due to a genetical shift in the mouse strain (the animals were heterozygotes, although from the same farm). It is to be emphasized that a shift occurred both in the response to exposure to X-rays as well as to adrenaline administration. This observation suggests the existence of some correlation between the response of the body to hormonal changes and the effect of X-rays as far as the resorption and distribution of labelled compounds is concerned. There remains considerable uncertainty concerning the mechanism of the interaction particularly because the sensitivity to ACTH-treatment was found to be quantitatively similar in the two instances whereas the X-ray and adrenaline effect were dissimilar. These findings have, however, a general implication in isotope and other studies as rapid resorption and distribution processes in the body may likely be influenced by the hormonal balance of the animal.

Summary

Exposure to X-rays, administration of adrenaline or ACTH were found to influence the rate of resorption of injected labelled bicarbonate by mice as indicated by the amount of $^{14}\text{CO}_2$ exhaled. When repeating the experiments half a year later, 2—3 times as high X-ray dose and a 5 times higher adrenaline dose had to be applied to obtain the same effect on $^{14}\text{CO}_2$ exhalation while no change in the sensitivity to ACTH was observed.

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86. EFFECT OF IRRADIATION WITH X-RAYS ON THE CATABOLISM OF METHYLALCOHOL IN THE MOUSE

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WATERY suspensions of numerous enzymes can promptly be inactivated, often by exposure to a restricted dose of ionizing radiation, as first shown by DALE (1942). By exposing a suspension of adenosine triphosphatase (myosin) to a dose of only 10 r BARRON (1949) observed a 10 per cent inactivation of this enzyme. This type of prompt inactivation of enzymes is not often met *in vivo*, possibly due to the presence of a great variety of protecting substances and a rapid whipping out of possible changes produced by the exposure. FORSSBERG (1945) did not succeed in inactivating catalase present in the liver, even when he applied as massive doses as 83,000 r. A few days after exposure, a progressive inactivation of catalase was, however, observed by FEINSTEIN *et al.* (1950) perhaps due to a blocking of the new-formation of metabolized catalase molecules. The half-lifetime of catalase molecules in the liver of the guinea pig was found to be 5 days (BONNICHSEN and THEORELL, 1951).

Enzyme inactivation can also be studied by administering a metabolite marked with ^{14}C and studying the amount of labelled CO_2 exhaled previous to and after exposure. If irradiation reduces the amount of labelled CO_2 exhaled, this does not prove an inactivation of the enzymes involved; viz. the effect of exposure may be due to other reasons than enzyme inactivation and so far the latter took place, it may be difficult to ascertain which of the enzymes involved in the catabolic process was influenced. If, however, irradiation does not affect the amount of exhaled CO_2 we can conclude that the enzyme system involved in the catabolic process was not influenced by exposure or, more correctly, that a sufficient fraction of enzymes involved remained intact and can thus perform its task at a normal rate. In the present note the results of a study of the effect of exposure of mice to irradiation with X-rays on the catabolism of methyl alcohol are communicated.

EXPERIMENTAL

Albino mice, weighing 14–16 gm, were placed each in one of six perforated aluminium boxes which were fixed in a plexy glass box of 1.5 litre volume. CO_2 -free air passed through the box carrying the exhalatory CO_2 which was absorbed by a set of centrifuge tubes containing saturated $\text{Ba}(\text{OH})_2$. 3 bottles each containing 50 ml solution proved to be sufficient to absorb all CO_2 . The centrifuged BaCO_3 precipitate was washed with CO_2 -free water and ethanol, dried first in a vacuum exsiccator, then by heating to 105° .

In one set of experiments, minute amounts ($3\text{ }\mu\text{gm}$) of methyl alcohol dissolved in 0.050 ml saline were administered by intraperitoneal injection to each mouse having an activity of $1.5\text{ }\mu\text{curie}$. In another set massive doses (8 mg) of methyl alcohol dissolved in 0.050 ml saline were injected. The exhalatory $^{14}\text{CO}_2$ of the same group of mice was collected first, previous to irradiation and again after the lapse of one day or more and also after exposure to a dose of 1500 r. In both cases methanol was injected in each experiment shortly before the collection of the exhaled CO_2 had started. As one day after the administration of $3\text{ }\mu\text{gm}$ of methanol the specific activity of the exhaled CO_2 was less than 1 per cent of that observed in the early stage of the experiment, repeating the experiment the following day the ^{14}C present in the exhalatory CO_2 originating from the first experiment could be disregarded. Even after administration of a massive dose of methanol the ^{14}C content of the exhalatory CO_2 declined in the course of 1 day to less than one per cent of the value observed in the early part of the experiment.

In contrast to the controls exposed mice could only be applied once. Before starting the irradiation, food but not water was withdrawn from the mice and from the controls at a corresponding time. In those experiments, in which the effect of exposure was not studied immediately after irradiation, but first after the lapse of 1 day, food was withdrawn 40 hr before starting the experiment, as the food consumed and resorbed by the exposed rats differs from that of the controls; even by pair-feeding this source of error can not be completely eliminated. The experiments were carried out in a thermostat room kept at a constant temperature of 27.1° . The control mice were kept in the same wooden box in which irradiation took place as were the exposed mice. As the amount of CO_2 exhaled shows a diurnal variation, the experiments had to be carried out always at the same hour.

The specific activity of the exhaled CO_2 was determined by comparing the activity of BaCO_3 samples of identical weight (100 mgm) with that of a known aliquot of the methanol injected after converting it into BaCO_2 .

RESULTS

The result of a set of experiments in which after injecting a small amount ($3\text{ }\mu\text{gm}$) of methanol, the animals were kept previous to the collection of the exhalatory CO_2 10 minutes in the apparatus, through which CO_2 -free air passed, is seen in Fig. 1. This figure shows also the results of a second experiment on mice freshly injected the next day; it gives furthermore, the data obtained the third day, when the same animal was exposed to a dose of 1500 r previous to injection of methanol.

The amount of exhaled CO_2 by the same group of mice is mostly showing quite appreciable variations, even if the exhalatory CO_2 is collected

at the same hour of the day and emotional disturbances of the animals are largely avoided. Trying to evade their cage, the animals perform muscular exercises which may result in an additional CO_2 formation.

In the experiment, the results of which are plotted in Fig. 1, the variations in the amount of exhaled CO_2 were unusually low; the 6 mice of 14.7 gm mean weight exhaled in the 3 experiments (two with controls and one with exposed animals) in the course of 5 min an average amount of CO_2 amounting to 34.9, 35.1 and 34.8 mgm, respectively.

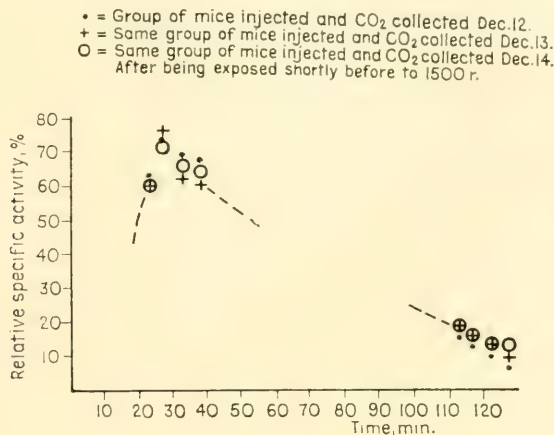


FIG. 1. Effect of exposure to 1500 r of X-rays on the amount of $^{14}\text{CO}_2$ exhaled by a group of mice injected with a "tracer dose" of labelled methyl alcohol.

After a maximum value, 21 minutes following the injection of methanol, the specific activity of the exhalatory CO_2 declines to 1/4 of that value in the course of further 77 minutes. In other experiments this decrease was found to take 73 to 81 minutes. After the lapse of 19 hours the specific activity declined to 0.3 per cent of its maximum value. As seen from Fig. 1 exposure to 1500 r did thus not influence significantly the combustion rate of small amounts of administered methanol.

The experiments described hitherto were carried out in an almost physiological milieu. We then administered large amounts of methanol, 8 mgm to each animal, thus creating strongly unphysiological conditions. These experiments were carried out to test whether a strong shift of the ratio substrates enzyme in favour of the substrate causes a depression of the combustion rate of methanol due to exposure of the animals to X-rays.

In one group of experiments we injected 8 mgm methanol to each mouse and collected the expiratory $^{14}\text{CO}_2$ for 5 hr. After the lapse of a day, the specific activity of the expiratory carbon declined to 2 per cent of the

maximum value observed. We then administered the same dose of labelled methanol and collected again the expired CO_2 for 5 hours. The third day the mice were exposed to 1500 r, then injected with methanol and the collection repeated. The second day the controls exhaled with 14 per cent more $^{14}\text{CO}_2$ than the first day; the third day a further

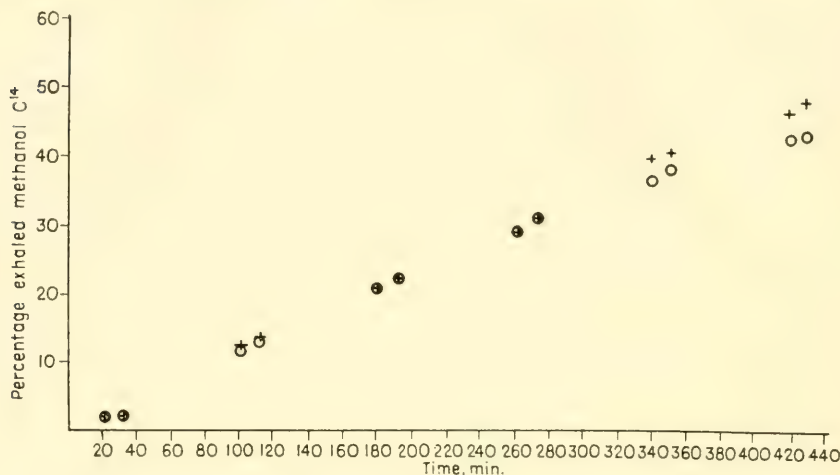


FIG. 2. Effect of exposure to 1500 r of X-rays on the amount of $^{14}\text{CO}_2$ exhaled by a group of mice injected with a massive dose of labelled methyl alcohol.

increase in the amount of $^{14}\text{CO}_2$ exhaled by 20 per cent was observed. In another set of experiments the exposed mice exhaled 14 per cent more $^{14}\text{CO}_2$ than did the controls.

In the experiments described above methanol was administered without delay after exposure. In the experiment the results of which are seen in Fig. 2, methanol was injected 24 hours after exposure to a dose of 1500 r. During the first $4\frac{1}{2}$ hr controls as irradiated mice exhaled the same amount of $^{14}\text{CO}_2$, in the later part of the experiment the irradiated mice gave off somewhat more radioactive carbon dioxide.

DISCUSSION

In the living organism methanol is oxidized to formaldehyde and on to formic acid (POHL 1893). Liver tissue (KEESER and VINCKE 1940), to a minor extent kidney and other tissues (BARLETT 1950) as well were found to oxidize methanol to formaldehyde. After the consumption of methanol relatively large amounts of formate are excreted in the urine (ASSER 1914).

BARTLETT studying the combustion of labelled methanol in the rat recovered 89 per cent of the administered ^{14}C , viz. 65 per cent as CO_2 in expired air, 14 per cent as methanol in the expired air, 3 per cent as methanol in the urine, 3 per cent as formic acid in the urine, and 4 per cent fixed in tissues. In his experiments the rat was given by stomach tube 1 mgm methanol per 1 gm body weight. The complete combustion as measured by radioactive CO_2 formation was found to proceed independently of the alcohol concentration at a rate of $25\ \mu\text{gm}$ per gm body weight per hr. In our experiments after intraperitoneal administration of 0.53 mgm of methanol per gm body weight it took 260 min to convert 30 per cent of the methanol carbon to CO_2 , the combustion rate being $37\ \mu\text{gm}$ per gm of body weight per hr. The mouse burns thus per g body weight one and a half times as much methanol as does the rat.

Very small amounts of methanol, $0.3\ \mu\text{gm}$ per gm of body weight, are not combusted at the same rate as larger amounts. At a rate of $37\ \mu\text{gm}$ per hr $0.3\ \mu\text{gm}$ should be converted into CO_2 in the course of less than a minute, during this interval the descending slope of the curve of Fig. 1 representing decreasing specific activity values of the exhaled CO_2 decreases only slightly. In the presence of minute amounts of methanol the rate of intrusion into the tissue is presumably the rate determining reaction.

While none of our experiments shows a depression of the combustion rate of methanol in the exposed mice, they show that possibly a slight increase in that rate takes place. In numerous cases was an increased rate of biochemical reactions observed in the exposed organism, i. e. by FORSSBERG, studying the effect of massive doses on the activity of catalase extracted from the liver or sarcoma of the rat, carcinoma of the rabbit or of the coleoptiles of growing barley. Similar results were obtained by us (EULER and HEVESY, 1942) when investigating the effect of exposure of rats on the activity of the catalase of their Jensen-sarcoma. That catalase accelerates the oxidation of methanol was found by BONNICHSEN and THEORELL (1951) and the slightly increased rate of methanol combustion in the exposed mouse may be due to an enhanced catalytic action of catalase as ascertained by FORSSBERG and others.

Summary

A massive dose of ^{14}C labelled methanol is converted into CO_2 by the mouse at a rate of $37\ \mu\text{gm}$ per gm of body weight per hour, thus one and a half times more rapidly than by the rat.

Neither the complete combustion of minute ($0.3\ \mu\text{gm}$ per gm body weight) nor that of large amounts ($530\ \mu\text{gm}$ per g of body weight) of methanol is depressed by exposure of the mice to an X-ray dose of 1500 r immediately or one day before injecting the animals.

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87. EFFECT OF IRRADIATION WITH X-RAYS ON THE CATABOLISM OF ETHYLALCOHOL IN THE MOUSE

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WATERY suspensions of numerous enzymes can promptly be inactivated, often by exposure to a restricted dose of ionizing radiation, as first shown by DALE (1942). This type of prompt inactivation of enzymes is not often met *in vivo*, possibly due to the presence of a great variety of protecting substances and a rapid whipping out of possible changes produced by exposure. FORSSBERG, for example, (1945) did not succeed in inactivating catalase present in the liver even when he applied as massive doses as 83,000 r. In those experiments, the splitting effect of liver slices of controls and exposed animals on hydrogen peroxide was compared and in the study of the effect of irradiation on other enzymes their effect on other suitable substrates.

Enzyme inactivation can also be studied in the intact animal by administering a metabolite marked with ^{14}C and studying the amount of labelled CO_2 exhaled previous to and after exposure. If irradiation reduces the amount of labelled CO_2 exhaled, this does not prove an inactivation of the enzymes involved; viz. the effect of exposure may be due to other reasons than enzyme inactivation and so far the latter took place, it may be difficult to ascertain which of the enzymes involved in the catabolic process was influenced. If, however, irradiation does not effect the amount of exhaled CO_2 we can conclude that the enzyme system involved in the catabolic process was not influenced by exposure or, more correctly, that a sufficient fraction of enzymes involved remained intact and can thus perform its task at a normal rate. In an earlier communication (1953) we found mice previously exposed to a dose of 1500 r not to catabolise methylalcohol at a lower rate as controls. In the present note the results of a study of the effect of exposure of mice to irradiation with X-rays on the catabolism of ethylalcohol are communicated.

EXPERIMENTAL

Albino mice, weighing 12—14 gm, were placed each in one of six perforated aluminium boxes which were fixed in a plexy glass box of 1.5 liter volume. CO_2 -free air passed through the box carrying the exhalatory CO_2 which was absorbed by

a set of centrifuge tubes containing saturated $\text{Ba}(\text{OH})_2$. 3 bottles each containing 50 ml solution proved to be sufficient to absorb all CO_2 . The centrifuged BaCO_3 precipitate was washed with CO_2 -free water and ethanol, dried first in a vacuum exsiccator, then by heating to 105° .

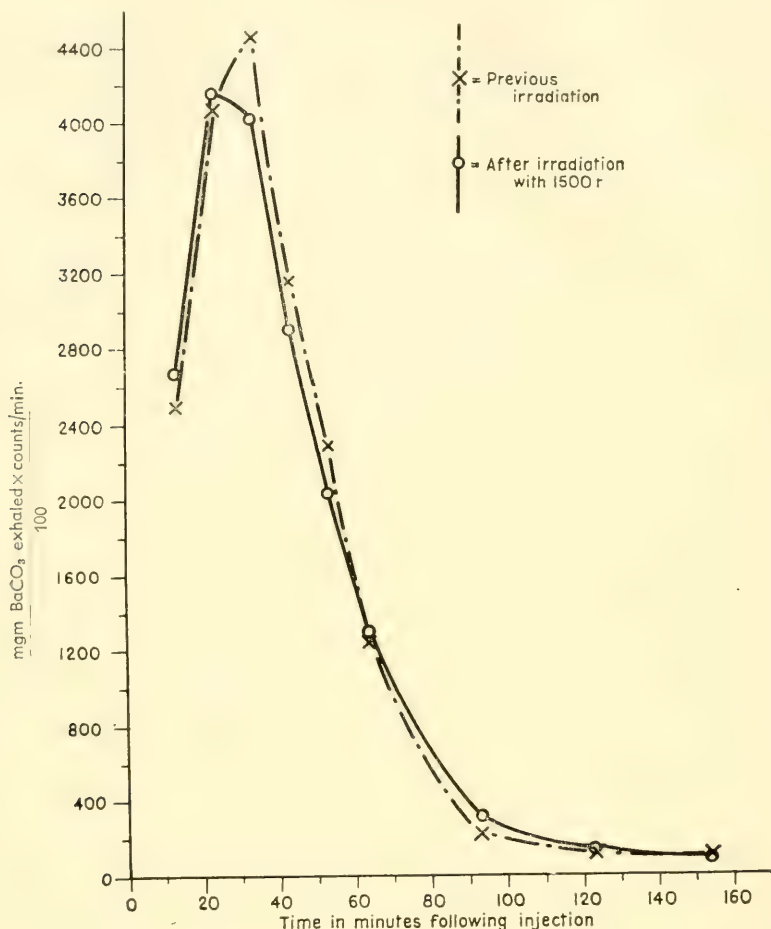


FIG. 1. Effect of irradiation on the exhalation of $^{14}\text{CO}_2$ by labelled ethyl alcohol injected mice.

To each mouse 0.050 to 0.100 ml of a 20 per cent ethyl alcohol solution labelled by addition of 1 to $3\mu\text{gm}$ alcohol- $1\text{-}^{14}\text{C}$ were administered by intraperitoneal injection. The exhalatory $^{14}\text{CO}_2$ of the same group of mice was collected at intervals of 10 min first, previous to irradiation and again after the lapse of two days and also after exposure to a dose of 1500 r. In both cases ethanol was injected in each experiment before the collection of the exhaled CO_2 had started, and within $\frac{1}{2}$ hr of the termination of irradiation which took 30 min. The Roentgen tube applied was run with 180 kV and 7 millamp. In some of our experiments the controls were injected in intervals of 2 days. This was made possible by the fact that the specific

activity of the exhaled CO_2 declined already after the lapse of 5 hr to less than 3 per cent of its maximum value observed in an early stage of the experiment.

In contrast to the controls exposed mice could only be applied once. Before starting the irradiation, food but not water was withdrawn from the mice and from the controls at a corresponding time. In other experiments, food was withdrawn 20 hr before starting the experiment, as the food consumed and resorbed by the exposed mice differs from that of controls; even by pair-feeding this source of error cannot be completely eliminated. The experiments were carried out in a thermostat room kept at a constant temperature of 27.1° . The control mice were kept in the same wooden box in which irradiation took place as were the exposed mice. As the amount of CO_2 exhaled shows a diurnal variation the experiments had to be carried out always at the same hour.

The specific activity of the exhaled CO_2 was determined by comparing the activity of BaCO_3 samples of identical weight (100 mgm) with that of the ethanol injected after converting it into BaCO_3 .

RESULTS

The result of a typical experiment, in which after 20 hr of fasting 6 mice were injected with labelled ethanol, whereupon food was given for about a day and the animals irradiated and injected again the second day after 20 hr of fasting, is seen in Fig. 1. The figures were obtained by plotting the product of the weight of BaCO_3 obtained by collecting the exhalatory CO_2 in baryte, and the activity of 100 mgm of that compound. As seen from the figure no significant difference is shown by the amount of $^{14}\text{CO}_2$ exhaled by the mice previous to and after irradiation. In 4 further experiments the total amount of $^{14}\text{CO}_2$ exhaled by the exposed animals differed by +14, -8, -7, +12 per cent from that given off before irradiation. When injecting the same group of mice 3 times without and the fourth time after exposing them to 1500 r we obtained the following relative figures: 100, 112, 109, 102 for the amount of $^{14}\text{CO}_2$ collected.

VITALE and assoc. (1953) investigated the rate of alcohol metabolism in rats administered varying doses of alcohol by using ethanol-1- ^{14}C and measuring the $^{14}\text{CO}_2$ excreted in the respired air. They found the rate of alcohol oxidation to be directly proportional to the amount administered up to a maximum of about 2.5 to 3 gm per kilo of body weight. When 0.9 gm alcohol/kgm body weight was injected to each rat they found the maximum rate of oxidation of ethanol to amount to 220 mgm/kgm hr. during the 2nd and 3rd hour after its administration. We found in our experiments with mice a corresponding value of 197 mgm/kgm hr. This result was obtained with animals fed till the start of the experiment. When mice that had been fasting for 20 hr were investigated, the corresponding figure was 149 mgm/kgm/hr.

That fasting animals catabolise ethanol at an appreciably lower rate than fed-ones was already observed by VITALE and assoc. While they recovered 70-90% of the alcohol administered to fed rats, the recovery

from fasting rats amounted to 35–93% only. BARTLETT and BARNET (1949, 1953) observed a recovery of 75% from fed rat in the course of 5 hr (cf. also DONTCHEFF (1950) and BURBRIDGE and HINE (1951)). Fasting was also found to decrease the ability of liver slices to oxidize ethanol (VITALE *et al.*, 1953). Ethanol was found most rapidly oxidized in such experiments, when its concentration in the surrounding medium was 25 mgm% (MASORO *et al.*, 1953).

Summary

The rate of oxidation of ethylalcohol by control mice and such exposed to a dose of 1500 r of roentgen rays was compared by administering with alcohol-1- ^{14}C labelled ethanol and collecting the $^{14}\text{CO}_2$ exhaled. No significant difference was found in the amount of $^{14}\text{CO}_2$ exhaled by the controls and the irradiated animals.

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COMMENT ON PAPERS 83–87

WHEN mice were injected with ^{14}C labelled glucose and the exhaled CO_2 + and $^{14}\text{CO}_2$ collected, the exposed animals were found to exhale less CO_2 and $^{14}\text{CO}_2$ than the controls (paper 83). The effect of Roentgen rays and hormones on the resorption rate of injected $\text{NaH}^{14}\text{CO}_3$ was studied with FORSSBERG as well (papers 84, 85). The mean life-time of the bulk of the circulating bicarbonate ions in the body of the mouse being some minutes only, any change in the rate of resorption will be reflected in a corresponding change of the $^{14}\text{CO}_2$ exhaled within a few minutes or even seconds after injection. Irradiation was found to depress the resorption rate, the exhalation of ^{14}C being reduced. ACTH treatment before irradiation annihilates the effect of the latter. Administration of adrenalin decreased the output of $^{14}\text{CO}_2$.

Neither the rate of catabolism of methyl alcohol nor that of ethyl alcohol was found to be influenced by exposure to irradiation (papers 86, 87), demonstrating the radiation resistance of the enzymes involved in the catabolic process.

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88. RADIOACTIVE TRACERS IN RADIOBIOLOGICAL STUDIES. THE THIRTY-SIXTH SILVANUS THOMPSON MEMORIAL LECTURE

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It is with a sense of great privilege that I am delivering this lecture to commemorate the great and first president of the Roentgen Society. Radiology is deeply indebted to Silvanus THOMPSON for the foresight, enthusiasm and energy with which he so successfully served its cause. Not alone the application of X-rays but many other branches of science and art benefited by the great vigour and breadth of his mind. Silvanus THOMPSON was my first teacher in Electricity, his book *Elementary Lessons on Electricity and Magnetism* my first text-book in that field. I am, therefore, all the more grateful to this Institute for the high honour it has done me in affording me the opportunity of paying a tribute to the man for whose personality and writings I have had a particular admiration since my early youth.

Glancing at earlier Silvanus THOMPSON lectures, great names and great presentations emerge. I have to restrict myself to the discussion of some applications of radioactive tracers in the study of pathochemical effects produced by ionizing radiation, effects which are early steps in those changes which the radiotherapist wishes to achieve.

Damage and destruction of cells are possibly the most important radiobiological effects. To the understanding of an important type of cell death in which mitotic processes are involved the application of radioactive tracers has materially contributed.

INTERFERENCE WITH THE FORMATION OF DESOXYRIBONUCLEIC ACID

Quite shortly after the discovery of artificial radioactivity by Frédéric and Irène JOLIOT-CURIE, radioactive phosphorus was applied first in the study of the renewal of the mineral constituents of the skeleton, then in that of the rate of the renewal of phospholipids and the acid-soluble phosphorus compounds present in the animal organism. A few years later we extended these studies to the investigation of the rate at

which labelled phosphate is incorporated into the desoxyribonucleic acid present in various tissues (HAHN and HEVESY, 1940). The nucleus of the cell being its most radiosensitive part and desoxyribonucleic acid, DNA, being one of the main constituents of the nucleus, it seemed to be pertinent to investigate if the rate of incorporation of phosphate into DNA is influenced by exposure to radiation. As phosphorus is one of the constituents of DNA, no DNA molecule can be formed without incorporation of phosphorus and thus, if we label the plasma phosphate by administering radioactive phosphorus with the food or by injection, the phosphorus utilised in DNA formation becomes labelled and ^{32}P is bound to enter the newly formed DNA molecule.

In early studies incorporation of ^{32}P into the Jensen-sarcoma of the rat was studied (EULER and HEVESY, 1942, 1944). Half of the 156 rats investigated were exposed to a dose of 335 to 1500 r. They showed a depression of ^{32}P incorporation into the DNA secured from the sarcoma compared with the incorporation into the DNA of the sarcomata of non-irradiated controls. A very similar result was obtained in studies carried out by HOLMES (1947, 1949).

When the activity of 1 mgm of DNA phosphorus of the Jensen-sarcoma of the rats exposed to total body radiation of 335 r or more is compared with the labelled ^{32}P content of the DNA of the Jensen-sarcoma of unirradiated rats, the former was found to be about 40 per cent only of the latter; thus the rate of DNA formation was depressed by exposure to less than half its normal value. Table 1 shows values of this depression obtained at different times after exposure of the rat.

TABLE 1

Dose in r	Time between exposure and injection of ^{32}P	Time between injection and killing of rat	Ratio of ^{32}P content of 1 mgm sarcoma DNA of controls and irradiated rats
750—1500	few minutes	$\frac{1}{2}$ hour	3.2
350—1000	few minutes	1 hour	2.4
1500	few minutes	4—6 hours	2.8
1230—1500	3—7 days	2 hours	1.7

Similar results were obtained by us (AHLSTRÖM, EULER and HEVESY, 1945) in the investigation of the formation of labelled DNA in the normal organs of 250 rats. In liver of 3.5 to 4.5-day old rats in which the percentage increase in the labelled, thus additionally formed, DNA was found to be 20 times larger than in the one-year-old animal; the percentage depression of ^{32}P incorporation due to irradiation differed only slightly from that observed in the outgrown animal. Very numerous investi-

gations, for example those by KELLY, HIRSCH, BEACH and PAYNE, (1955) carried out later, much extended these findings.

One may ask why radioactive indicators had to be used to arrive at the above-mentioned results. In the course of one hour the increase in the mean DNA content of the Jensen-sarcoma of our rats amounted to 0.75 per cent. If exposure should depress the rate of formation by 10 per cent the determination of this difference by the usual analytical methods would necessitate the measurement of a change of 0.075 per cent in the DNA content of the sarcoma. This is, even today, an almost insurmountable task, although in the course of the 16 years which have elapsed since the experiments were carried out great progress has been made in the field of the analytical chemistry of nucleic acids.

If we apply radioactive tracers the determination of a change in the rate of formation of DNA becomes an easy task. We have now only to take into consideration the 0.75 per cent of labelled DNA molecules, and taking these to be 100, a depression in the radioactivity from 100 to 90 is quite easy to determine.

If a popular comparison may be permitted, if we had to identify a criminal by investigating the affairs of every inhabitant of this city, this would be an almost insurmountable task, which would be immensely facilitated if Scotland Yard could restrict its investigations to persons registered in its annals, thus to labelled ones.

Calculation of the Amount of DNA Formed

While it is quite easy to determine if and to what extent exposure to irradiation influences incorporation of ^{32}P into DNA, and thus the rate of formation of new DNA molecules, the quantitative determination of the amount of DNA formed involves appreciable difficulties. The calculation from radioactive data of the amount of DNA formed during a time interval necessitates the knowledge of the precursor of the DNA phosphorus and its specific activity. The simplest assumption to make is that the administered radioactive phosphate penetrates into the tissue cells, gets uniformly mixed with the orthophosphate present in these and is then utilised in the synthesis of DNA. If 1 mgm of such phosphate during the experiment has a mean activity of 100 relative units, and if at the end of the experiment taking one hour, 1 mgm of DNA phosphorus has the activity of one, then 1 per cent of the DNA molecules present must have been built up in the course of one hour.

Experience shows, however, that orthophosphate is far from being always the precursor of the organic phosphorus compounds present in the cell. In early investigations (HEVESY, BARANOWSKI, GUTHKE, OSTERN and PARNAS, 1938) it was found, for example, that when adenyphosphate is formed from adenosine in the presence of non-labelled

hexosediphosphate and labelled inorganic phosphate in yeast, one-half of the phosphorus atoms present in the adenyolphosphate were those originally located in the hexosediphosphate molecules, while the other half only were originally present as orthophosphate. Later, when investigating the formation of phospholipids in the liver, it was observed that it is the glycerophosphate phosphorus which is incorporated into phospholipids (CHAIKOFF, 1942) and as the formation of labelled glycerophosphate is a comparatively slow process, formation of phospholipids after administration of ^{32}P proceeds first for a while from inactive glycerophosphate. Thus, for a while, phospholipid formation is not indicated by the radioactive tracer and correspondingly, when calculating its rate from the specific activity of the cellular orthophosphate P and the lipid P we underrate the rate of the formation of the phospholipids. When calculating the rate of formation of DNA as described above, the possibility must therefore be envisaged that the ratio

$$\frac{\text{specific actiivty of DNA P}}{\text{mean specific activity of orthos phosphate P}}$$

supplies the lower limit of the formation of DNA only.

Incorporation of ^{14}C Into DNA

Results similar to those obtained when we were following up the effect of irradiation on the incorporation of ^{32}P into DNA were obtained when the effect of exposure on the incorporation of ^{14}C into DNA of the organs of the mouse was investigated. When ^{14}C became available we injected growing mice with labelled sodium acetate ($\text{CH}_3\text{.}^{14}\text{C}\text{OONa}$) and compared the rate of incorporation of ^{14}C into the DNA of the organs of animals exposed to 700 r of X-rays with that of non-exposed mice (HEVESY, 1949). As can be seen in Fig. 1, exposure depresses the incorporation of ^{14}C into DNA of the intestinal mucosa, liver, muscles, and kidney of growing mice to about one half of that of controls. Incorporation of ^{14}C into proteins, however, is not depressed by irradiation: it is even somewhat enhanced.

In early investigations on radiation effects MITCHELL (1940, 1942), applying ultraviolet micrography used in conjunction with photographic photometry, observed a striking increase in ultraviolet absorption of the cytoplasm of proliferating and incompletely differentiated irradiated tumour cells due to the accumulation of ribonucleotides. These early investigations and many subsequent ones revealed that irradiation with ionizing radiations influences ribosenucleic acid formation as well.

The influence of radiation on the rate of incorporation of precursors into ribosenucleic acid (RNA) was often found to be much less marked than in DNA, HOLMES (1949) being the first to make this observation.

The ratio of the rate of incorporation of ^{32}P or ^{14}C into DNA and RNA in the exposed organism shows appreciable variations. These depend on the nature of the precursor, the time left for the labelled compound to take part in the metabolic steps involved and the dilution of the newly formed labelled DNA or RNA by "old" non-labelled ones present. As the

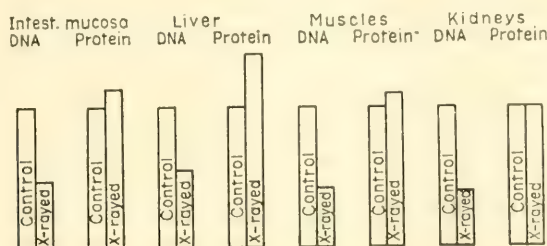


FIG. 1. Effect of irradiation, 700 r X-rays, on the incorporation of ^{14}C of injected $\text{CH}_3^{14}\text{COONa}$ into tissue fractions of the growing mouse.

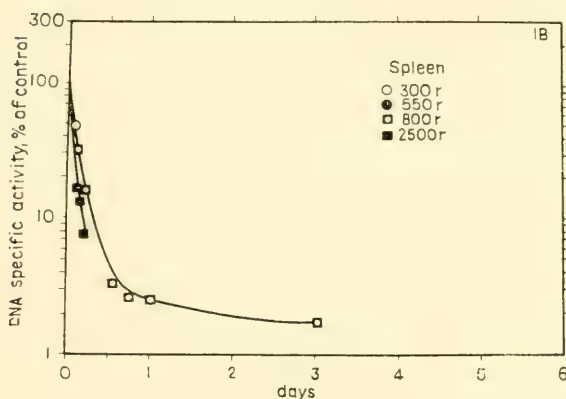


FIG. 2. Effect of irradiation on the incorporation of ^{32}P into the DNA of the spleen of the rat (KELLY *et al.*)

total RNA content shows greater variations with time than that of the DNA content, the dilution effect will show a more intricate pattern in the case of RNA than in that of DNA. The great difference in the synthetic rate of nuclear and cytoplasmic PNA will also contribute to make the effect of irradiation on the incorporation of ^{32}P or ^{14}C into PNA much more dependent from the experimental conditions than that into DNA. While the effect of irradiation on PNA formation is mostly less pronounced than on DNA formation, this interference may have very far reaching consequences as well.

Investigation of the Effect of Exposure to Radiation on Unicellular Organisms

Investigation of the effect of irradiation on enzyme activity *in vivo* led to the result that damage to the cell presumably mainly precedes inactivation of its enzyme. Enzymes which are found to be refractory in the radioresistant liver can easily be damaged in the radiosensitive spleen. Oxidizing phosphorylation of mitochondria of the thymus, which can be affected by a dose of 50 r, remains unaffected in the liver even after exposure to massive doses (VAN BEKKUM, 1956). In view of these

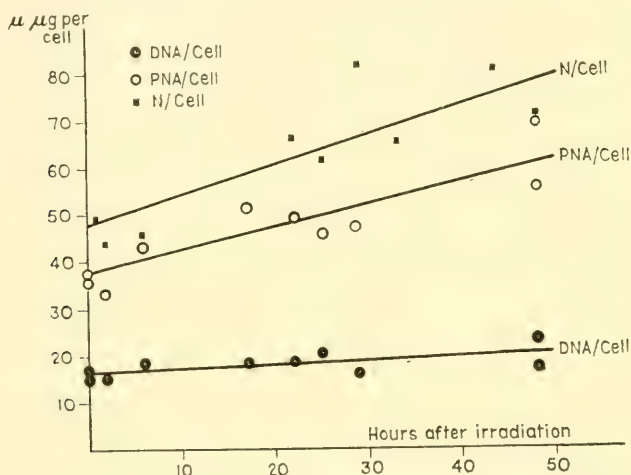


FIG. 3. Increase of the average amount of PNA, DNA and total nitrogen per ascites tumour cell after irradiation of the mouse with 1250 r.

facts one may be tempted to interpret interference with DNA synthesis to be a sequel of general cell damage as well. This is presumably often the case. In bone marrow cultures, where incorporation of ^{32}P and ^{14}C into DNA is immediately stopped after a 5000 r exposure taking 15 minutes (LAJTHA and SUIT, 1955), cell damage presumably preceded interference with DNA formation. The same applies to the observations made by HOLMES (1954) who found that exposure of the rat to 450 r only slightly depressed ^{32}P incorporation in DNA of regenerating rat-liver, while a massive dose of 2000 r produces an immediate 50 per cent depression. That ^{32}P incorporation into the DNA of the spleen of rats exposed to heavy doses, a radiosensitive organ in which cell destruction due to irradiation is very pronounced, is almost entirely absent is demonstrated by Fig. 2 (KELLEY, *et al.*, 1955). Studying the effect of exposure of mice to 1250 r on the incorporation of ^{14}C of glycine or adenine into DNA of ascites tumour cells, FORSSBERG and KLEIN (1953), however, observed a marked interference of ^{14}C incorporation into DNA in these unicellular

organisms, though their vital staining revealed that they were living all through the 48 hours period of the experiment. These and other observations prove that interference with DNA synthesis does not necessarily involve visible cell damage.

FORSSBERG and KLEIN observed, as can be seen in Fig. 3, an increase in the total nitrogen and PNA content of the exposed ascites tumour cells with time in contrast to their DNA content, and the same result is demonstrated by Fig. 4, which is taken from a paper of KELLNER

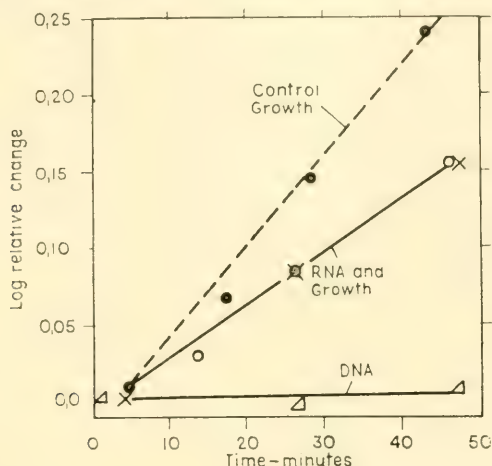


FIG. 4. Effect of irradiation with ultraviolet light on growth and formation of constituents of *E. coli*.

(1953) who irradiated *E. coli* with ultra-violet light. Following the change in the protein, PNA and DNA content of these bacteria, he found the formation of the last mentioned compound to be blocked after exposure, but not the formation of the first mentioned ones. Irradiation by ultra-violet radiation thus affects DNA formation immediately. Thirty minutes after irradiation with Roentgen rays was the shortest time interval after which ^{32}P incorporation into the DNA of rat sarcoma was investigated (cf. Table 1), a very pronounced effect being observed.

As mentioned on p. 855 in the experiments described, irradiation did not depress the incorporation of ^{14}C into cellular proteins. Nor was it found to depress the incorporation of ^{35}S into nuclear proteins of the root of *Vicia faba* (HOWARD and PELC, 1951).

Accumulation of cellular constituents

The marked interference with one of the main processes, the formation of DNA going on in the course of the mitotic cycle, is one of the most conspicuous radiation effects. Other cell constituents accumulate and

having no opportunity, cell division being obstructed, to be distributed between the mother and a daughter cell, the cell swells. When the cell recovers its DNA synthesizing and dividing capacity it may divide, but the cell being abnormal this process often has lethal consequences. That exposed cells often die when trying to divide has been known for many years and was emphasised, for example, by LEA in his classical book *Actions of Radiations on Living Cells*.

As shown by HOWARD and PELC (1953) exposure to radiation can also interfere with mitosis in a stage of the mitotic cycle in which synthesis of DNA is already terminated. Thus we meet a second type of mitotic interference which may lead to cell death or to the formation of abnormal cells.

In a recent investigation of FORSSBERG (1956), during the first two post-irradiation hours the incorporation of glycine-2- ^{14}C into DNA of ascites tumour cells was found to be reduced to 70 per cent of that into non-irradiated controls. In the following amitotic period no further reduction in the specific activity of DNA took place. A slight increase in these values was in fact observed. It is well-known that after moderate doses the division of such cells as are in a sensitive stage gets retarded while those in a less sensitive stage proceed through their cycle at a normal rate. This may result in a piling-up of cells which were to enter mitosis. When at a later stage the cycle of the last-mentioned cells sets in again, the mitotic index increases as compared with unirradiated material. No indications of an increased mitotic activity were found in FORSSBERG's experiments, but steps preceding such an activity may have taken place involving slightly increased DNA formation. It is the radiation effect mentioned above which does not involve interference with DNA formation that may have prevented the manifestation of the mitotic process.

Autoradiographic studies

Our knowledge of the part of the mitotic cycle in which synthesis of DNA takes place and of the radiation sensitivity of various parts of this cycle was very much enlarged through the work of HOWARD and PELC (1951, 1953 and 1956) who introduced autoradiographic methods in the study of ^{32}P incorporation into DNA present in the meristem cells of main roots of *Vicia faba* seedlings. They found the normal mitotic cycle in the meristem to take 30 hours, of which four are spent in division, 12 between the end of division and the beginning of ^{32}P uptake into new DNA, six in DNA synthesis as judged by ^{32}P uptake, and eight between the end of synthesis and the beginning of division. The number of cells synthesizing DNA is reduced by exposure to 50 r to about 60 per cent of the normal value during the subsequent 12 hours. Increase of the

dose to 150 r does not increase the percentage of cells not DNA synthesizing. The results are interpreted by them to be due to a greater radiosensitivity, to delay or inhibition of DNA synthesis on the part of cells which are in approximately one third of the cell cycle (the first

part of interphase) at the time of irradiation. The marked effect of exposure to a 140 r dose of (190 kV) X-rays on the uptake of ^{32}P by DNA of such cells is seen in Fig. 5.

Results very similar to those of HOWARD and PELC were recently obtained by LAJTHA, OLIVER and ELLIS, (1954) in investigations of the incorporation of added orthophosphate- ^{32}P into human marrow cultures or adenine- ^{14}C into DNA in the course of the intermitotic cycle. The total cycle time was found to be of the order of 40 to 48 hours for the average dividing bone marrow cells. DNA synthesis took place during 12 to 15 hours in the second half of the cycle only and was divided from the mitosis by a three to four hour non-synthesizing period, as is seen in Fig. 6.

The important results obtained by HOWARD and PELC and LAJTHA, OLIVER, ELLIS and SUIT help us also to understand why exposure to radiation often depresses DNA formation to one to two thirds of that taking place in the non-irradiated tissue

only. In such cases where exposure to radiation interferes to a minor extent only or does not interfere at all with DNA synthesis, as in the case of refractory tumours, the above explanation clearly does not suffice. We shall revert to this point.



FIG. 6. The cell cycle to DNA synthesis, measured by the incorporation of ^{32}P or adenine- ^{14}C into DNA.

Interference with the DNA molecule

In view of the complexity of the processes involved it cannot be stated with certainty, but it is very probable, that irradiation does not block or mainly block the formation of intermediates of DNA formation

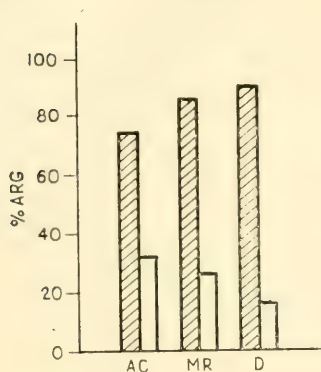


FIG. 5. Percentage of cells showing autoradiograph after 140 r X-rays followed by 24 hours of ^{32}P . Hatched columns: unirradiated controls.

Empty columns: unirradiated. AC=All cells of *Vicia faba* in the final 2 mm of the root. MR=Meristematic resting nuclei. D = Dividing cells.

but rather interferes at some later stage in the biosynthesis of desoxy-ribopolynucleotides (ERRERA, 1954).

The DNA molecule has the largest weight, about 6×10^6 , of all compounds found in the mammalian organism. Its length can be estimated to be about 3×10^{-4} cm. A molecule of such length has a very much larger probability of picking up energy in the exposed organism and of leading it to a radiosensitive spot than a small one. WATSON and CRICK (1953) have given arguments for assuming that the DNA molecule

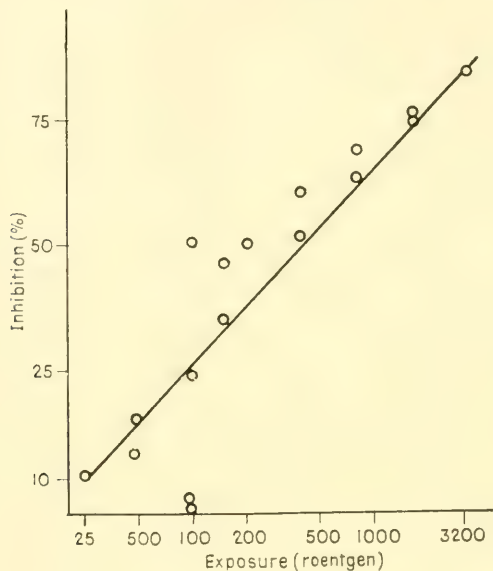


FIG. 7. Incorporation of ^{32}P into rat thymus DNA after X-irradiation.

consists of two polynucleotide chains wound helically in a large number of turns around a common axis and tied together by hydrogen bonds between corresponding purines and pyrimidines. To explain the duplication process of the desoxyribosenucleic acid molecule, WATSON and CRICK suggested that the two polynucleotide chains may be separated and each chain catalyses the synthesis of a complementary chain.

Different modifications of this scheme were put forward, all of which suggested that the multiplication of the DNA molecule takes place in a template fashion and necessitates the presence of undisturbed DNA molecules. By accepting this view we arrive at the conclusion that an interference with the DNA molecules present may block new formation of such molecules and that the observed effect of irradiation on DNA formation may be a consequence of the disturbance of the DNA molecules or of the protein moieties of the nucleoprotein which have to serve as a template in the process of additional formation of such molecules.

Desoxynucleoproteins are not necessarily reproduced in a template fashion. CHARGAFF (1955) is inclined to consider the adjoining nucleotide rather than the one opposite to direct incorporation. In this as well as in the first mentioned case breaking of bonds may suffice to interfere with the formation of new nucleotide molecules.

Breakage of hydrogen bonds may produce extensive damage. Breakage of one hydrogen bond requires about 10^{-20} calories. The average energy of an ionization being $32 \text{ eV} = 1.25 \times 10^{-19} \text{ gm calories}$, a single ionization

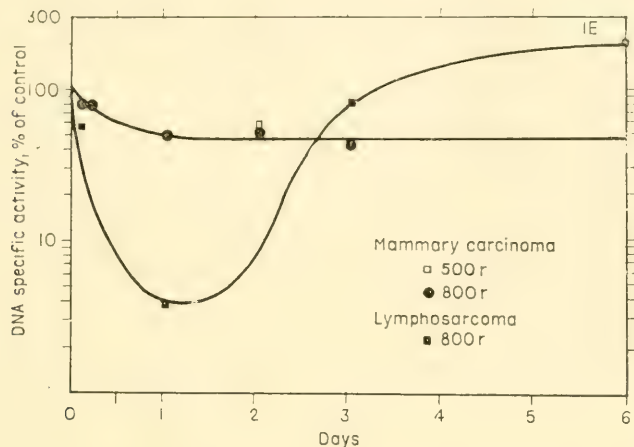


FIG. 8. Effect of exposure on the incorporation of ^{32}P following intraperitoneal injection of labelled sodium phosphate.

1, capable of breaking 100 hydrogen bonds (FRANK and PLATZMAN, 1955; POLLARD, GUILD, HUTCHINSON and SETLOV, 1955). BUTLER (1956) states that an energy of 18 MeV suffices for the breaking of a phosphate bond in dry DNA, which may lead to changes going deeper than the breaking of a hydrogen bond. He considers the possibility that the DNA-histone bond is affected and that the breakage of a comparatively few histone-protein bonds may prevent DNA synthesis.

Numerous and very extended studies were carried out on the effect of radiation on DNA or DNA nucleotide suspensions. The viscosity of suspensions was measured prior to and after exposure to irradiation, and various other methods were applied as well. BUTLER (1956), for example, reports that while 15 minutes of heating at 100°C had very little effect on the sedimentation constant of DNA, after a dose of 9000 r it becomes much more sensitive to action of heat.

In early investigations a dose of thousands of Roentgens had to be applied to obtain a marked depolymerisation of the DNA (SPARROW and ROSENFELD, 1946; TAYLOR, GREENSTEIN and HOLLAENDER, 1947)

or DNA protein suspension (ERRERA, 1947). Recently, ANDERSON has shown that in fresh rat thymus homogenates containing only 50 μgm of DNA in 1 ml. 1 M NaCl solution viscosity is reduced with time after exposure to as little as 50 r of γ or X-rays. With 500 r a marked reduction was apparent at once, with an almost complete loss of viscosity after four hours. The results obtained in fresh homogenates exposed to 150 r much resemble those arrived at when purified preparations of DNA were exposed from 50,000 to 100,000 r, a coincidence which supports the view that the native DNA exists in the form of molecules of extreme length while the purified samples are composed of relatively small molecules. It demonstrated also the very significant fact that large molecules are more easily damaged by irradiation than small ones.

In experiments with young rats ORD and STOCKEN (1956) found a dose of 25 r to suffice to reduce ^{32}P incorporation into DNA of the thymus at two hours by 10 per cent, the percentage damage increasing proportionally with the dose, as seen in Fig. 7. A restricted dose thus interferes both with the formation of DNA in the thymus nuclei of the exposed animal and with the DNA present in the exposed diluted homogenate. This damage may open the way for interference with the normal enzymatic pattern with all its far-reaching consequences.

That a few days after the exposure of the adult rat to about 500 r the DNA content of the thymus is found to be reduced to half of its initial value (HARRINGTON and LAVIK, 1955) may be at least partly due to the suppressed replacement of the lymphocytes. In the liver, as to be expected, no loss of DNA was observed. DNA prepared from livers of rats irradiated with 1000 r after intervals of one day and two days was found to be very similar to that prepared from unirradiated livers (BUTLER, 1956).

That enzymes present in radiosensitive organs can be interfered with by applying restricted doses, not, however, such present in less radiosensitive organs, may be a consequence of the easier vulnerability of the desoxyribosepolynucleotides in the former. It was shown recently by FORSSBERG (1956) that the concentration of lactate, citrate, acetoacetate and other cell constituents is strongly influenced by irradiation in the ascites tumour cells of the mouse. As soon as the faculty of the cell to synthesis DNA recovers, the above mentioned concentration changes cease as well. In the liver, which is much less radiosensitive than the thymus, the concentration changes of metabolites were found to be absent.

In *in vitro* experiments, DALE (1956) observed that an energy of 32.5 eV absorbed sufficed to oxidize 40 to 60 cysteine molecules to cystine, and the liberation of sulphur from thiourea can reach values of 90. These are large yields due to chain reactions. We do not usually observe similar effects in *in vivo* experiments as the radiation energy, due to the pre-

sence of a great variety of protecting substances, is prevented from reaching the enzymes present in the tissue. By producing cell damage, for example through lesion of the DNA nucleotides, the normal enzymatic pattern gets changed and chains of pathochemical effects may set in as in the cases mentioned above.

Radiosensitivity

A correlation between radiosensitivity and vulnerability of desoxyribopolynucleotides, between radiosensitivity and macro-molecular lesion of DNA, to use an expression introduced by MITCHELL (1956), is so far a hypothetical one, not so the correlation between radiosensitivity and rate of formation of DNA. It is easy to find deviations from such a correlation but as is demonstrated by the data in Table 2, in a great number of cases a striking correlation is found between radiosensitivity of the organ and the turnover rate of its DNA. Table 2 shows the percentage of DNA formed in the organs of the adult rat in the course of 24 hours. The sequence of radiosensitivity is that stated by WARREN and BOXERS (1950). This is in spite of the fact that radiation sensitivity depends, in addition to genetically determined factors, on cellular composition, on the metabolic state of the cell, on the rate at which precursors and humoral agencies reach the cell and waste products are removed, and on other factors. When BERGONIÉ and TRIBONDEAU pointed out 50 years ago that radiosensitivity increases with decreasing age and decreasing differentiation of the cell and with increasing mitotic figure they made a most pertinent observation.

TABLE 2. — TURNOVER FIGURES

DNA turnover per cent per day	Organ	Radiosensitivity in decreasing order
90	Bone marrow	Lymphocytes Erythroblasts Myeloblasts
50	Intestinal mucosa	Epithelium of intestinal crypts
42	Thymus	Thymus
24	Spleen	Spleen
1.6	Muscle tissue and connective tissue	Connective tissue
0.8	Liver	Liver
0.4	Kidney	Kidney
0.4	Brain	Nerve and brain
0	Nucleated erythrocytes	Nucleated erythrocytes

One of the most conspicuous exceptions from this correlation is the great radiosensitivity of lymphocytes in spite of the almost total absence of mitotic action. It is, however, of interest to recall the fact mentioned above that the DNA of lymphocytes of the thymus is depolymerised under the action of a very restricted radiation dose. Furthermore erythrocytes, though very refractory towards exposure to radiation, may be damaged by haemolysing agencies accumulating in the irradiated organism. Such agencies may affect the fragile lymphocytes much more pronouncedly.

Radiosensitivity of plant seed may differ by a factor of 10 or more, whereas their growth rate per time unit, and consequently DNA formation, do not much differ. The composition of seeds may strongly differ and in different seeds a very different fraction of radiation may reach the sensitive spots of the mechanism responsible for the synthesis of DNA.

The radiosensitivities of higher plants show great variation as well. The lethal dose for acute radiation varies from less than 1000 r for *Podophyllum peltatum* to a dose in excess of 200,000 r for *Cratogeomys*. SPARROW (1955) suggested that the high radiation tolerance of certain cruciferae or of *Gladiolus* might be due to a high content of vitamin C or other reducing substances. Thus, these plants exert self-protection by removing damaging oxidizing radicals and reducing oxygen tension.

Another example that marked turnover rate of DNA is compatible with high radioresistance is the existence of refractory tumours. Even massive doses cannot interfere with the formation of DNA in these. Fig. 8, taken from a paper of KELLY *et al.* (1955) demonstrates that while ^{32}P incorporation into lymphosarcoma ceases almost fully for a day or so following an exposure to a dose of 800 r, incorporation into the investigated mammary carcinoma is hardly influenced.

Numerous types of DNA exist. DNA of a given cell was found by CHARGAFF (1955) to be composed of a very large number of differently constituted individuals. From the Walker carcinoma two DNA fractions were isolated, the ratio of which markedly changed after exposure to a dose of 5000 r (HARBERS and BACHMANN, 1956). Different types of DNA having different radiosensitivities, refractory tumours may be those in which DNA of small radiosensitivity predominates. The radiosensitivity of tumour cells may, however, be determined to a large extent by conditions prevailing in their environment.

That the presence of oxygen strongly enhances radiation damage is a well established fact (GRAY, 1953a). In the presence of oxygen more damaging radicals are formed from the irradiated tissue water, which transmit much of the radiation energy. Furthermore the effect of irradiation depends on the metabolic activity of the cell during irradiation

which in turn is influenced by the amount of available oxygen (LASER, 1954). GRAY, CONGER, EBERT, HORNEY and SCOTT (1953) that observed the sensitivity of the ascites tumour cells of the mouse to X-rays is about three times as great when irradiated in a well-oxygenated medium as it is under anoxic conditions. This observation may prove to be of great importance to the radiotherapist.

HAEMOPOIETIC ARREST

Anaemia is the classic radiation disease. In the healthy man daily 0.85 per cent of the circulating red corpuscles complete their life-cycle and have to be replaced. Radiation interferes with the formation of red corpuscles and may accelerate their disappearance from the circulation as well. It takes days before the anaemia can be ascertained by counting of the red corpuscles, determination of the corpuscle volume or the haemoglobin content of the blood. By following the effect of incorporation of radioiron into haemoglobin of the circulation or that present in the bone marrow, the haemopoietic arrest produced can be ascertained at an earlier date, in the rat by investigating the ^{59}Fe content of the circulating haemoglobin, after six hours. Only a few minutes after intravenous injection of ^{59}Fe a slight amount of radioiron can be detected in the haemoglobin of the circulation amounting in man to 0.3 per cent after the lapse of one hour (DAL SANTO, 1956). This activity, however, is presumably due to the incorporation of ^{59}Fe into the haemoglobin of the circulating reticulocytes.

HENNESSY and HUFF (1950) were the first to show that the rate of appearance of administered radioactive iron (^{59}Fe) in the circulating red corpuscles was considerably depressed by even small doses of whole body radiation given 24 or 48 hours before the injection of iron. This result was repeatedly confirmed. As can be seen in Fig. 9, which is taken from a paper by BAXTER, BELCHER, HARRISS and LAMERTON (1955), the exposure of rats to a dose of 24 r already has a marked effect on the incorporation of subcutaneously injected ^{59}Fe in the haemoglobin content of the circulating blood, and thus on haemoglobin formation.

The depressed rate of formation of haemoglobin could be due to an interference with the biochemical synthesis of haemoglobin or of haemin in the erythropoietic marrow cells, or alternatively to a mitotic interference or a lesion of the latter. If haemin synthesis *per se* would be radiosensitive we would expect the formation of other haemins present in the organism, that of myoglobins, catalases, cytochromes, to be depressed as well after exposure of the animal to radiation. This line of thought induced us to follow the effect of irradiation on the incorporation of ^{59}Fe , not only into haemoglobin, but also into the above mentioned

haemins of guinea pigs, rats and rabbits exposed to 500 r, and in some cases up to 1400 r, and killed 5 to 66 hours after the injection of radio-iron (HEVESY and BONNICHSEN, 1955; BONNICHSEN and HEVESY, 1955).

The results of these experiments are seen in Fig. 10. ^{59}Fe incorporation into haemoglobin and myoglobin alone is interfered with by exposure

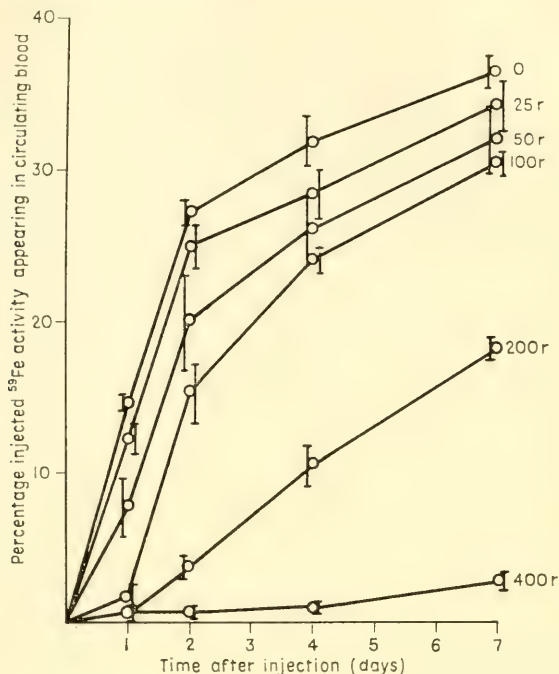


FIG. 9. Uptake of ^{59}Fe in circulating blood of rats ("August" strain) injected subcutaneously with ferric chloride in citrate buffer 48 hours after irradiation with various doses of 250 kVp X-rays.

to radiation. Incorporation of ^{59}Fe into all other haemins investigated is not depressed; it is even enhanced. As after irradiation the number of marrow cells, the chief competitors for ^{59}Fe , get strongly reduced, more ^{59}Fe takes its way into the cells of the liver and other organs.

Doses up to 100 r do not disturb clearance of ^{59}Fe in man (LOEFFLER, COLLINS and HYMAN, 1953), but a dose of 800 r was found to decrease markedly the clearance of ^{59}Fe in the rabbit (HEVESY and DAL SANTO, 1954).

The above findings suggest the explanation that interference with haemoglobin formation is due to the radiation sensitivity of marrow cells. Radiation produces mitotic arrest in the latter, new-formation of erythropoietic cells is interfered with and, if larger doses are applied, cell destruction independent of mitotic processes may take place as well.

As long as incomplete erythropoietic cells are present the laying down of haemoglobin into these is going on which follows from the fact that it takes one to two days before the full effect of the haemopoietic arrest gets visible by a depressed ^{59}Fe content of the circulating haemoglobin.

Exposure of the mouse to 400 r leads after 30 minutes to a depression of the mitotic figure of the erythrocytic series of the bone marrow to one fourth of its normal value (KNOWLTON and WIDNER, 1950).

Cell destruction

In tadpoles exposed to 500 r haematopoietic cell destruction was found to be directly correlated with the diminished rate of cell division during the same time (SCHJEIDE and ALLEN, 1951). When, however, massive



FIG. 10. Effect of X radiation on incorporation of ^{59}Fe .

doses of 10,000 to 20,000 r were applied, the rate of haematopoietic cell destruction surpassed that of cell division, indicating cell destruction independent of mitotic processes. LASNITZKI (1943), exposing tissue cultures of avian fibroblasts to 1000 r of X-rays of low ionization density, could not observe degenerated cells 30 minutes after exposure; but when she exposed the tissue cultures to 1000 rep of β -rays, having a high ionization density, she could observe 3 per cent degenerated cells immediately. These degenerated cells were due to an immediate effect both on mitotic and resting cells.

Cell death may be entirely disconnected from mitotic processes and also from interference with the DNA molecule. This is shown among

other by the fact that the physiological death rate of the mammalian red corpuscle not containing DNA may be accelerated by exposure of the organism to radiation. Irradiation reduces their life-cycle possibly partly through production of agencies promoting haemolysis.

Interference with the rate of blood flow is another example where processes independent from cell division promote cell death. DOBSON

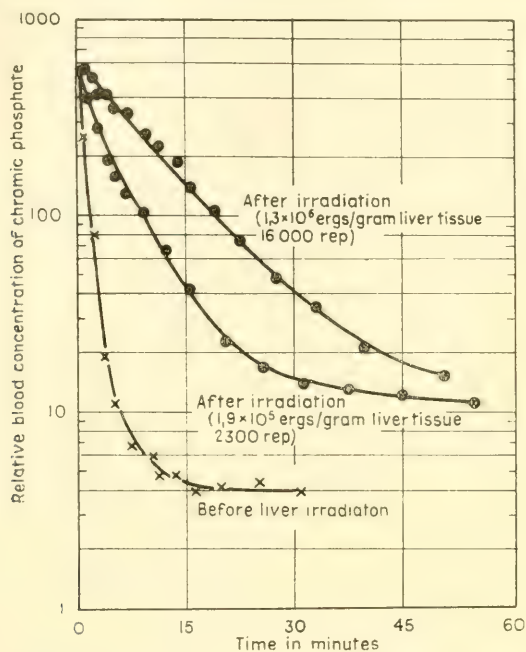


FIG. 11. Changes in the rate of chromic phosphate disappearance with liver irradiation in the rabbit.

and JONES (1951) followed the rate of disappearance of colloidal chromic phosphate labelled with ^{32}P from the circulation of various animals and found it markedly depressed after exposure to 2300 r (cf. Fig. 11). The depression observed is at least partly due to a diminution of the rate of liver blood flow.

That it is a cytological damage which is responsible for the interference with haemoglobin formation was shown by making use of the autoradiographic technique, thus applying a direct method by LAJTHA and SUIT (1955) and SUIT, LAJTHA and OLIVER (1956). They studied incorporation of ^{59}Fe into both irradiated and non-irradiated human bone-marrow cells *in vitro*, and into bone marrow of the rabbit *in vivo*. The application of the autoradiographic method led, as was mentioned on page 859, in the hands of HOWARD and PELC to very important results. Striking results were also obtained by LAJTHA and SUIT. They could demonstrate very convincingly that although the early cell forms of the nucleated red

corpuseles of the human marrow are more radiosensitive than the late forms, those cells which survive radiation for 16 to 24 hours show no impairment in their ability to take up ^{59}Fe *in vitro*. Nor did irradiation of rabbits with 100 to 1000 r affect the uptake of ^{59}Fe by the cells. They state, in accordance with the results discussed above: "It appears that the extreme radiosensitivity (of haemoglobin synthesis) shown *in vivo*

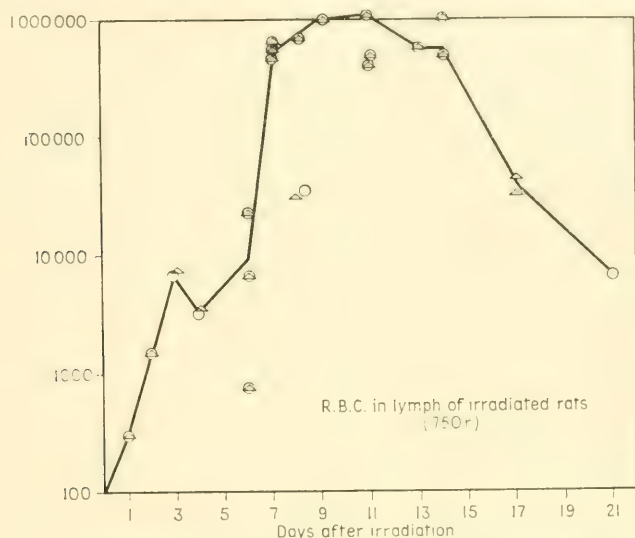


FIG. 12. Erythrocyte counts in the lymph of rats exposed to 650–750 r of X-rays.

is more likely to be due to impairment of the rate of erythrocyte formation (mitosis) than to the impairment of haemoglobin synthesis."

In addition to interfering with the formation of red corpuseles and shortening the life-time of circulating erythrocytes, irradiation may make the capillary wall permeable to erythrocytes. These leak out into the interspaces (WISH, FURTH, SHEPPARD and STOREY, 1952), and into the lymph flow (ROSS, FURTH and BIGELOW, 1952) as can be seen in Fig. 12, and soon get haemolysed. Thus the candle is burned at both ends. No new red corpuseles are formed which should replace those which terminated their life-cycle and even some of those circulating can leak out. The untightness of the capillary wall is presumably due to thrombopenia, as a minimum platelet level seems to be necessary to keep the capillaries in a physiological state. Radiation thrombopenia is due to the same causes as the haemopoietic arrest and as far as interference with mitotic processes is responsible for the latter, this interference is responsible for thrombopenia as well.

Exposure of dogs to 500 r was found to lead within three days to a mean decrease of the red corpusele volume by 12 per cent. Erythropoietic

arrest can explain a decrease of not more than 5 per cent in the course of three days (SOBERMAN, KEATING and MAXWELL, 1951). If the additional decrease is due to haemolysis produced by irradiation or to leakage through the capillary wall cannot be stated.

By making use of radioiron as an indicator, extended studies were carried out by LAMERTON and his colleagues (cf. BAXTER *et al.*, 1955) on the pattern of recovery from radiation damage and how this is influenced by shielding procedures. They demonstrated *inter alia* that anaemia observed in animals given 450 r with one hind limb shielded is less severe than in animals given the same dose of whole body radiation; the bone marrow of the shielded limb shows a high degree of erythropoietic activity two to three days after irradiation. The spleen of a shielded animal becomes actively erythropoietic much earlier than in an unshielded animal. The hind-limb shielding is almost as effective in lessening the severity of the anaemia in the splenectomized as in the intact animal.

GROWTH PROMOTION

Determination of biochemical reaction rates with great accuracy may reveal that every such reaction is influenced in some way by exposure to radiation. In numerous cases which time prevents me from discussing even in part, such influences were ascertained. The great radiation sensitivity of the DNA molecule and its formation, the far-reaching consequences of the radiation damage produced by such an interference, and the important role radioactive indicators played in revealing the depression of DNA formation under the effect of exposure to radiation induced me to discuss this type of radiation damage more in detail.

By making use of radiophosphorus as a tracer it could also be demonstrated that growth going on in one organ may promote DNA formation in other organs. KELLY, LOLA, PAYNE, WHITE and JONES (1951) investigated the incorporation of ^{32}P into DNA of the organs of mice bearing bilateral transplants of mammary carcinoma (cf. also CERECEDY, LOMBARDI, REDDY and TRAVERS, 1952; SMELLIE, MCINDRE and LOGAN, 1953). As can be seen in Fig. 13, incorporation of ^{32}P into DNA of the liver of the mouse, and thus DNA formation in that organ, is promoted by the presence of mammary carcinoma, ^{32}P incorporation increasing with the age of the tumour transplant. ^{32}P incorporation into DNA was also found to be promoted in the spleen and kidney but not in the intestinal mucosa of mice with mammary carcinomas.

KELLEY and JONES (1953) found furthermore that DNA turnover can be increased in livers and spleens of normal mice by repeated injections of various fractions of homologous tissue mashes and also of cell-free extracts of such mashes.

When Ehrlich tumour cells irradiated *in vitro* were inoculated into mice and the cell multiplication was assayed, results were obtained by FORSSBERG (1956) which suggested that decay products from X-ray killed and lysed cells may serve the survivors as an additional substrate, thus enhancing the growth rate. Forssberg emphasised the importance of transfer of metabolites from dead to living cells. While these and possibly also humoral substances promote DNA formation, exposure to radiation exerts a contrary effect. Twelve hours after exposure of mice

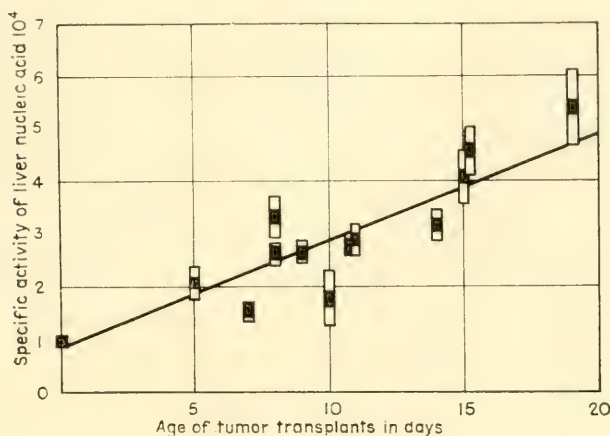


FIG. 13. The relationship between age of tumour and specific activity of liver desoxypentosenucleic acid in mice bearing mammary carcinomas.

to 1040 r the specific activity of the liver DNA phosphorus of mice injected with embryo mash was found by KELLEY and JONES (1953) to be 0.29 while that of non-injected animals was only 0.19.

The early observation that while irradiating a flank of the tumour bearing rat the incorporation of ^{32}P into DNA of a second protected tumour present in the other flank of the rat was depressed as well is presumably to be explained in a similar way (AHLSTRÖM, EULER and HEVESY, 1947; HOLMES, 1949).

From the above results it follows that presence of a tumour, even if metastases are absent, promotes growth of some organs.

The facts which I have had the privilege to communicate were to some extent intermingled with hypotheses. A hypothesis, as the late Sir J. J. THOMPSON remarked, is not a creed but a policy. How far the hypotheses mentioned will, if only on a very modest scale, prove to be useful in promoting the understanding of pathochemical changes produced by radiation the future has to reveal.

In November 1897, SILVANUS THOMPSON delivered the first Presidential Address to the Roentgen Society. It was devoted to the study

of the discovery of X-rays and to their consequences. He remarked that "it is clear that our little Society has an abundant field before it to engross the activities of its members, not only for the approaching winter, but many years to come". Fifty-nine winters have passed since these words were spoken. They are still valid, and may be expected to be valid for a great number of winters to come.

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COMMENT ON PAPER 88

In paper 88 it is emphasized that in most cases the radiosensitivity of an organ is proportional to its DNA turnover. Already half a century ago Bergonié and Tribondeau observed a connection between the radiosensitivity of an organ and its mitotic figure. As mitosis is preceded by DNA formation, a connection as the above mentioned one has to follow from Bergonié and Tribondeau's consideration. The measurement of the rate of formation of labelled DNA will often be preferred to the counting of mitoses.

DNA turnover indicates mostly cell turnover. If the latter is rapid, as in the case of bone marrow, for example, little time will be at disposal to replace the destroyed cells and we shall find the organ to be radiosensitive. In the resting liver cells, dying per day make out less than 1/100 of those dying in the bone marrow. Replacement is correspondingly less exacting in the resting liver than in the bone marrow and the radiosensitivity of the liver lags much behind that of the bone marrow.

The radiosensitivity of the liver cell has not necessarily to differ from that of the marrow cell. The rapidly growing liver with its high DNA turnover rate is radiosensitive. We found in paper 72 the turnover rate of the liver of a 4 days old rat to amount to 40 times that of the fully grown animal.

Non-dividing lymphocytes are the most conspicuous example in which radiosensitivity is not going hand in hand with a high DNA turnover. Ord and Stocken consider it probable that the difference between dividing cells and lymphocytes may be attributed to the part which the cytoplasm, and particularly the mitochondria, can play in restoring or by-passing damage to the nucleus. In a dividing cell the nuclear damage can prevent multiplication and, if large exposures are used, disturbances in the mitochondria can prevent any reversal of the nuclear disorders, although with smaller exposures mitochondrial alterations are slight. In the non-dividing lymphocyte the volume of cytoplasm and number of mitochondria are very small. Nuclear metabolism is therefore a major factor in the economy of the cell, and its inhibition can have serious consequences because of the inability of the cytoplasm to overcome the damage.

The work of Howard and Pelc on bean-root tips and of Lajtha and his colleagues with human bone marrow cultures had indicated that synthesis of DNA occurs during a limited period of the mitotic cycle. Irradiation with ionizing radiation even after this period, which in bone cells amounts to 12 hours, is terminated and the full DNA complement reached, may stop the mitotic process. The depression of DNA synthesis following exposure to ionizing radiation does not necessarily indicate a radiosensitivity of the DNA formation but may be due to an indirect effect of the above types. The conclusion, however, occasionally met that the effect of irradiation on DNA formation is always due to such an indirect effect can hardly be maintained. Lajtha and associates studied the dose response curve of X-rays on synthesis of DNA in human marrow bone cells and also on the mouse Ehrlich ascites tumour cells during a 4-hour incubation period during which all the cells investigated were in their period of synthesis of DNA. Their experiments brought out that up to 4½ hours after a dose of 2000 rads synthesis of DNA is not stopped but produced at a reduced rate, thus less labelled desoxyribose nucleic acid is collected per cell during unit time. The decreased rate synthesis does not become

apparent until $\frac{3}{4}$ —1 hour after irradiation. The rate of synthesis of DNA was found to depend on two components, S_1 and S_2 . As to the nature of the S_2 component Lajtha and assoc. propose that it represents the integrity of the desoxyribonucleic acid template. The importance of the template for DNA synthesis is most spectacularly brought out by the work of Kornberg showing that the template has to be present to achieve *in vitro* synthesis of DNA. S_1 is interpreted as a part of the biochemical mechanism of precursor synthesis.

To a very similar conclusion arrive Ord and Stocken studying the effect of exposure to radiation on rat thymus desoxyribonucleic acid. They suggest that the initial steep portion of the dose—response curve corresponds to inhibition of nuclear phosphorylation and that the phase of only slowly increasing inhibition can be associated with progressive damage to the template.

Hagen has recently shown that DNA may be extracted from tissues with 90% phenol in the presence of trichloroacetate. He found that after exposure of rats to irradiation with X-rays (2 hr after 200—800 r) a higher yield of DNA can be obtained at the extraction with 1% trichloroacetate from the thymus tissue as well as from isolated thymusnucleohistone. If calcium ions are added to the nucleohistone of irradiated animals, the raised yield of DNA decreases again to normal values. A dose of 200 r suffices thus to influence the template of DNA synthesis.

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89. THE ABSORPTION AND TRANSLOCATION OF LEAD BY PLANTS

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THE investigation of the absorption of lead by plants can be carried out quite simply by dipping them into a solution which contains a radioactive isotope of lead, and determining the radioactivity of the ash from various parts of the plant. In addition to its simplicity and the extraordinary rapidity with which the work can be carried out this method possesses the following advantages: (*a*) By mixing suitable amounts of ordinary lead with the radioactive lead isotope, one can vary the lead concentration of the solution, as it were, between very wide limits. The assimilation of lead from a $N/1$ solution can be just as readily investigated as that from a solution many million times more dilute. (*b*) One can follow the change in localisation of the lead taken up by the plant, and thence draw conclusions as to the nature of its combination.

The experiments described in this paper were so carried out, that the plants, which had been cultivated in a culture solution, were washed with distilled water, and then the roots were immersed from 1 to 48 hours in a solution containing a mixture of lead nitrate and thorium *B* nitrate. In most cases *Vicia faba* (horse-bean) was used. After this period of immersion the individual parts of the plant were first well-rinsed with distilled water, and then ignited, and the intensity of the radioactivity of the ash was determined by means of an electroscope. This latter magnitude gives directly the lead content of the ash and thus also that of the corresponding part of the plant, when we know the radioactivity and the lead content of the solution in which the plant has been immersed.

Thorium *B* is a transformation product of thorium emanation, and is obtained in a very simple manner. A piece of platinum foil is charged negatively to a potential of 110 volts, say, and suspended in a vessel containing the preparation (radio-thorium, thorium *X*, etc.) from which the thorium emanation is generated. Under these circumstances the thorium *B* collects on the platinum surface, and can be removed with the aid of a few drops of dilute nitric acid. The normality as regards lead of a

solution (thorium *B* is an isotope of lead, i.e. a substance showing completely the chemical properties of lead) prepared in this way is about 10^{-12} , and if we wish to increase it we only need add to the solution a known amount of lead nitrate. For example, if we assume that we have prepared in this manner a 10^{-6} *N* solution of lead nitrate, and that after evaporating it to dryness it shows a radioactivity of 10,000 relative units, then each relative radioactive unit would correspond to an amount of $2 \cdot 10^{-5}$ mgm. of lead. We must of course take account of the fact that the material of the ash of the parts of the plants absorb part of the rays from the contained thorium *B*, but we can easily eliminate this disturbance by mixing the preparation used for comparison with the same quantity of ash as is contained in the sample the radioactivity of which we desire to know.

The following example shows the procedure during an experiment: *Vicia faba* that had undergone cultivation in a nutrient solution for a fortnight was introduced, after careful washing, into 500 cm³ of a 10^{-5} *N* radioactive solution of lead nitrate, which also contained 1/200 mol. of sodium nitrate. The temperature was 17°. The usual precautions such as screening the roots from light, etc., were also attended to. After 22 hours the plant was removed from the solution, and after careful washing with distilled water, the various parts—root, fruit, stem and leaves—were dried separately, ignited after the addition of a drop of concentrated sulphuric acid, and measured electroscopically⁽¹⁾. The result of the experiment is shown in the following table.

<i>Experimental (a).</i> Part of plant	Weight of ash in mgm	% of the total lead in the solution contained in the ash	Mgm of lead in the ash	Lead content of the ash in %
Roots	45	13.1	0.11	0.25
Fruit	5	0.10	0.0008	0.016
Stem	46	0.05	0.0004	0.001
Leaves	36	0.013	0.0001	0.0003

The purpose of the following experiments was to investigate the manner in which the assimilation of lead in the case of *Vicia faba* varies with the lead concentration of the solution. In all of these experiments the volume of the lead solution was 200 cm³ and the duration of the experiment was 24 hours.

The following collection of results shows that the individual experiments can be repeated, the agreement being quite satisfactory. Per cent

⁽¹⁾ Before the the measurement one must wait about six hours in order to be certain that radioactive equilibrium has been established between thorium *B* and thorium *C*. The reasons for this are outside the scope of this paper.

<i>Experiment (b). With 10^{-4} N lead solution</i>				
Part of plant ⁽¹⁾	Weight of ash in mgm	% of the total lead in the solution contained in the ash	Mgm of lead in the ash	Lead content of the ash in %
Root	41	60.0	0.02	0.052
Stem	12.6	0.04	0.000013	0.0001
Leaves	5.5	0.004	0.000001	0.00002
<i>Experiment (c). With 10^{-5} N lead solution:</i>				
Root	43	31.7	0.11	0.26
Stem	18	0.015	0.0004	0.002
Leaves	9.8	0.0012	0.00003	0.0003
<i>Experiment (d). With 10^{-3} N lead solution:</i>				
Root	39	11.9	3.9	10
Stem	18	0.02	0.007	0.04
Leaves	18	0.002	0.0007	0.004
<i>Experiment (e). With 10^{-1} N lead solution:</i>				
Roots	26	0.30	9.9	38
Fruit	18	0.11	3.6	20
Stem	11	0.065	2.2	20
Leaves	10	0.035	1.2	12

⁽¹⁾ The fruit was removed when the plant was introduced into the culture solution, since it constitutes a particularly good nutritive medium for troublesome moulds.

of the lead content taken up by the root from a 10^{-6} N solution: 61.2, 62.3, 57.4, 59.6, 55.4, 57.8, 47.3, 62.2, 61.7, 62.6, 60.0, 51.2, 68.7, 57.6.

From the above experimental data it is seen that, whereas in the case of a 10^{-6} N solution more than half of the lead is taken up by the root, the percentage loss when a 10^{-1} solution is used only amounts to 0.3, although the quantities of lead taken up by the root in the latter case are very much greater than in the former case. It is of interest to note that the *percentage* of lead which passes over into the stem and leaves from the concentrated solution of lead is not smaller than that from dilute solutions. This can be interpreted as meaning that with very dilute solutions the root itself is able to bind almost the whole quantity of lead, and thus renders extremely difficult the ascent of lead into the stem and leaves. On the other hand, when a concentrated lead solution is used, an ample sufficiency of unbound lead is available, and this can be carried upwards by the transpiration current. Except in the case of concentrated solutions, the root thus protects, as it were, the remaining parts of the plant, and this marked ability for "binding" lead is probably connected with an explanation of the relatively small toxicity of lead for plants, discussed on p. 444⁽¹⁾ [cf. STRASBURGER, 1891].

⁽¹⁾ Trees placed in solutions of copper sulphate or picric acid, etc. do not die until the poisonous substance has reached the highest points of the crown.

ON THE MODE OF COMBINATION OF LEAD IN THE ROOT

The question as to whether the assimilated lead enters into an organic molecule, or whether it is retained by the plant in the form of a saline compound can easily be decided. In the first case, lead atoms which had once been taken up by the root would not be able to interchange places with other lead atoms, whereas in the second case an active kinetic interchange between the lead atoms bound in the plant and those present in the solution would necessarily take place.

In order to make the argument clearer, we shall designate the atoms of lead in molecules such as those of lead tetraphenyl as "red" ones, and those which occur in such a form as lead nitrate as "blue" ones. If we dissolve both compounds in the same solvent and then separate them by crystallization, we should find only *red* atoms in the lead tetraphenyl and only *blue* ones in the lead nitrate, since the lead atoms in the lead tetraphenyl are available in an undissociable form. If, on the other hand, we dissolve equi-molecular amounts of lead chloride (with *red* lead) and lead nitrate (with *blue* lead), *i.e.* two salts in the same solvent, then after separation the two compounds would be composed half of *red* and half of *blue* lead atoms [HEVESY and ZECHMEISTER, 1920]. The distinction between *red* and *blue* corresponds here to radioactive and to inactive lead.

If the root has taken up active lead and we place it in a solution of inactive lead, then, if the active lead lies stably embedded in organic molecules, no active lead will be able to pass over into the solution, or in other words we shall not be able to displace the active lead with the aid of inactive lead. Now experiment shows that, with the help of a solution which is relatively rich in lead ($10^{-2} N$), we can remove almost quantitatively the lead taken up by the root, whence we must conclude that *the lead in the plant root exists in the form of a dissociable saline compound*, perhaps attached to the cell walls.

For example, if we introduce a *Vicia faba* (after careful rinsing) which has stood 24 hours in 200 cm³ of an active $10^{-6} N$ lead nitrate solution into a much more concentrated $10^{-2} N$ inactive lead nitrate solution of the same volume, we find that 95% of the active lead taken up by the root passes over into the $10^{-2} N$ solution; *i.e.* the active Pb-atoms are almost completely displaced from their places in the root by inactive atoms, which, of course, preponderate strongly (about 20,000 times), from the statistical viewpoint.

Now a $10^{-2} N$ lead nitrate solution is partially split up hydrolytically, and one might be inclined to ascribe the inverse dissolving action of lead nitrate to its acid content. However, with the aid of a $10^{-3} N$ HNO₃ solution it was possible to remove only 29% of the lead content of the root, and by the use of distilled water as solvent only 18% could be

removed. The investigation of the assimilation of lead from solutions of different lead content showed that from 10^{-4} N HNO_3 64%, and from 10^{-3} N HNO_3 practically the same amount, viz. 62% is taken up by the root, when the normality of the lead ions in the solution is 10^{-6} N . From a 10^{-2} N HNO_3 solution, a concentration sufficient, in general, to kill the plant, only 26% is assimilated by the root.

THE DISPLACEMENT OF THE LEAD TAKEN UP BY THE ROOT BY OTHER IONS

Since it has been established that we can displace the lead taken up by the root by other lead atoms, it seemed to be of interest to investigate the ability of other ions to displace the assimilated lead. 10^{-2} N solutions were used throughout these experiments. The plant containing lead was placed for 24 hours in the solution under consideration and then both the amount of lead remaining in the plant and the amount displaced into the solution were determined. The results of these experiments are shown in the following table:

Solution used	% of the lead initially present in the root which remained after treatment
Lead nitrate (inactive)	
Cupric nitrate	5
Cadmium nitrate	34
Zinc nitrate	38
Chromium nitrate	43
Barium nitrate	74
Sodium nitrate	76

When the reverse solution took place with the help of a 10^{-3} N $Pb(NO_3)_2$ solution, 14% of the originally assimilated lead were still present in the root after 24 hours' treatment.

Only copper is able to displace lead in a similar degree to lead itself. all the other cations investigated show an appreciably smaller displacing power.

The extent of the re-solution of the lead taken up by the stem and leaves was not determined. Experiments which are being undertaken on the assimilation of lead by *algae* will, amongst other things, also serve to shed light on this point.

As is well-known, different ions are assimilated to quite different degrees by plants, according to what other ions are present in the culture solution. The toxicity of individual types of ions is also arrested by others.

One of the best known cases of this "antagonism" is probably that between CaCl_2 and NaCl . In this case the phenomenon of the suspension of the toxicity of NaCl by CaCl_2 is attributed to the ability of the CaCl_2 to alter the plasma-membrane in such a way that it is less permeable to NaCl [OSTERHOUT, 1912]. Since it has been possible to show that in the case of lead a kinetic displacement of the assimilated ions by other ions occurs, we shall certainly have to reckon with the possibility that the antagonism is in individual cases occasioned by such kinetic effects.

LEAD ASSIMILATION AND TRANSPIRATION CURRENT

From the fact that more than 50% of the lead is taken up in 24 hours by the root in very dilute solutions of lead, i.e. a quantity of lead which was present in more than 100 cm^3 of liquid, we can conclude that it is not the *transpiration current* which transmits the assimilated lead, since the daily loss of water of *Vicia faba* under present conditions is less than 1 cm^3 . This independence is also shown in the following experiment. In one case the percentage assimilation of lead by the root was determined in the usual way, and in another after the root had been first separated from the stem under water. The volume of water was 500 cm^3 , the lead concentration was $10^{-6} N$, and $p_H = 4$. Duration of experiment = 1 hour.

Root as usual	6.8 and 7.5%.
Root cut off	6.5 and 7.1%.

It is seen that the amount of lead taken up was in both cases the same. Moreover, the lack of dependence of the assimilation of salt on the absorption of water by the plant has repeatedly been established [cf. ARRHENIUS, 1922].

THE TOXICITY OF LEAD

In connection with the experiments described in the previous section it is of interest to note that, as has been shown by BONNET [1922], the introduction of plants into $10^{-1} N$ $\text{Pb}(\text{NO}_3)_2$ solution unfavourably influences the transpiration current. In contrast to more dilute solutions, such an appreciable concentration of lead shows distinct toxic effects on the plant⁽¹⁾. *Vicia faba* which had stood 24 hours in a $10^{-1} N$ $\text{Pb}(\text{NO}_3)_2$

(1) Cf. also LAVISON [1911] and older experiments of PHILIPS [1883], KNOP [1885], NOLLE, BÄSSLER and WILL [1884].

solution already showed a slight deviation from the geotropic direction, and the leaves situated closest to the root showed signs of withering.

The toxic action of lead on different plants, such as wheat, radishes, lentils, cabbage, etc., has been investigated quite recently by BONNET [1922]. Just as in the present case, he introduced the plants into 200 cm³ of water after their roots had attained a length of several centimeters. The water contained in solution a definite amount of lead acetate or lead nitrate, and he obtained the following results:

(1) After the plants had stood in 10^{-1} *N* solutions of lead salts, lead could readily be detected qualitatively in the root.

(2) Only traces of lead were found in the stem and in the leaves.

(3) 10^{-1} *N* solutions of lead killed, e. g. the wheat plant after 20 days, balsam after two days.

(4) Mg, Ca and K showed no antagonistic action to lead.

(5) The greater the dilution, the less lead was taken up by the plant.

Our present results confirm these of BONNET. As regards the first result, we were able, thanks to the sensitiveness of the radioactive method, to detect with ease and quantitatively to determine the presence of lead even in the stem and in the leaves. It is interesting to note that Mg, Ca and K, which do not have an antitoxic action, have only a slight capacity for displacing lead, according to the experiments of the present author. In reference to point (5), the radioactive methods enable us to carry out a quantitative investigation of the dependence of the assimilation of lead on the concentration of the solution within wide limits, in which all other methods fail. In this manner, it is found that only 1/500 part of the amount of lead is taken up from a 10^{-6} *N* solution as compared with a 10^{-1} *N* solution. Those experiments of Bonnet should be mentioned, from which we can see the influence of the assimilation of lead on the growth of plants. He finds the following values:

Plant: The bean.

	Length of root in mm.		
	Initially	After 1 week	After 1 month
In water	25	100	1000
In 10^{-3} <i>N</i> Pb(NO ₃) ₂	31	31	32

Summary

(1) The assimilation of lead from lead nitrate solutions by *Vicia faba* has been investigated. A radioactive isotope of lead was mixed with the lead nitrate, and the amount of lead taken up was determined after ignition from the radioactive intensity of the ash of the various parts. This method makes possible the determination of exceedingly small amounts of assimilated lead.

(2) Whereas 0.3% of the lead is taken up by the root from 200 cm³ of a 10⁻¹ *N* lead nitrate solution in the course of 24 hours, 60% of the lead content of a 10⁻⁶ *N* solution is taken up in the same time. The leaves show a lead content of only a few hundredths or thousandths of 1% of the amount of lead present in the solution.

(3) The assimilated (radioactive) lead can be displaced by introduction of the plant containing lead into another lead solution, whereby inactive lead atoms now take the place of the radioactive ones. From this it follows that most of the lead is not combined with carbon within the plant, but that it exists in the form of a dissociable salt which is soluble with difficulty.

(4) Even after 24 hours, a 10⁻¹ *N* solution of a lead salt produces toxic effects on the plant, whilst more dilute solutions do not. Lead belongs to the least poisonous of the heavy metals.

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90. ATOMIC DYNAMICS OF PLANT GROWTH

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SOME years ago, one of us investigated the circulation of lead in plants using a radioactive lead isotope as indicator. Recent developments in nuclear chemistry make it possible to prepare radioactive isotopes of several of the light elements which, in contrast with lead, are the chief components of plant tissue. This enables us to investigate whether the atoms building up the tissues of the plant remain in their places or migrate during growth to other parts of the plant.

Let us, for example, consider the phosphorus atoms present in the lowest leaf of a maize plant; during the growth of the plant a second leaf will appear and the question to be decided is whether the first leaf gives up its own phosphorus atoms partly or wholly to build up the second leaf, or whether the phosphorus atoms carried up through the 'stem' (in reality undeveloped leaves) from the soil or from a culture solution are responsible for the formation of the second leaf. To solve this problem, the method of isotopic (radioactive) indicators can be applied using as indicator the radioactive isotope of phosphorus $^{32}\text{P}_{15}$, which has a half-life of about a fortnight. We prepared this isotope by bombarding carbon disulphide, in which a few milligrams of phosphorus were dissolved, with neutrons from a mixture of beryllium and a few hundred millicuries of radon, distilled off the carbon disulphide, oxidized the remaining phosphorus, and converted the phosphoric acid formed into sodium phosphate.

Small maize plants were grown for ten days in a culture solution of the usual composition and were then transferred to another culture solution in which the ordinary phosphorus was replaced by an equivalent amount of 'radioactive' phosphorus. Since the phosphorus isotopes cannot be separated by chemical processes, it follows that if, for example, one per cent of the radioactivity added to the culture liquid is found to be present in any part of the plant, we can conclude that one per cent of the those phosphorus atoms which were present initially in the culture solution are present also in that part of the plant. We will call the last mentioned phosphorus for the sake of brevity 'radioactive phosphorus'.

Time of the experiment (days)	Weight of ash (mgm)	Weight of the total P (mgm)	'Radioactive' P (mgm)	'Radioactive' P Total P
3.8				
Stem	53.8	2.05	0.806	0.395
Leaf	32.9	1.12	0.358	0.318
7.0				
Stem	44.0	1.39	0.878	0.630
Leaf	33.0	1.16	0.528	0.445
13.8				
Stem	136.5	5.74	4.21	0.733
Leaf	52.9	1.95	1.31	0.672
21.0				
Stem	157.9	5.45	4.31	0.791
First leaf	34.3	0.903	0.64	0.709
Second leaf	197.6	7.17	5.01	0.698

If we now determine by both chemical and radioactive analysis the phosphorus content of, for example, ash obtained after ignition of a leaf, we can determine how much of the total phosphorus present originated from the culture solution, that is, the "radioactive", phosphorus', and how much was already present in the plant at the beginning of

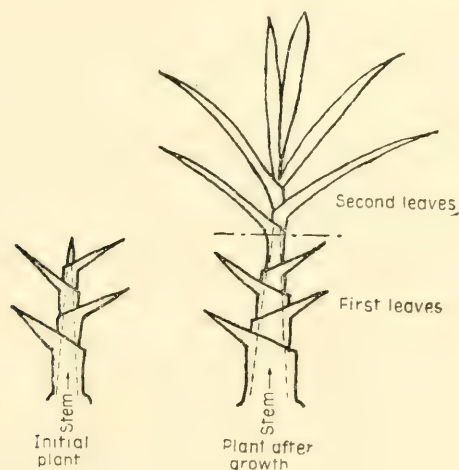


Fig 1.

the experiment. The result of the chemical and the radioactive analysis is seen in the accompanying table.

The chemical analysis was carried out by the colorimetric method described by LOHMANN and JENDRASIK⁽²⁾, and the radioactive analysis by comparing the activity of the ash of leaf or stem with that of a known

part of the radioactive phosphorus applied. In the last experiment the first and the second sets of leaves seen in Fig. 1 were ignited after the plant had been growing for 21 days in a culture liquid; the stem was also ignited. 79 per cent of the phosphorus content present in the stem was found to be 'radioactive' phosphorus originating from the culture solution, while the remaining 21 per cent was part of the ordinary phosphorus already present in the plant at the beginning of the experiment. For the lower and the higher leaves the corresponding figures are 71 and 70 per cent respectively 'radioactive' phosphorus and 29 and 30 per cent respectively ordinary phosphorus.

The practically identical ratios of radioactive phosphorus, originating from the culture liquid, to ordinary phosphorus, in the first and the second sets of leaves clearly indicate that the phosphorus atoms of the leaves are present in a mobile state, and that during the growth of the plant a continuous interchange of phosphorus atoms takes place between the different leaves. The data in the table show also three intermediate stages in the phosphorus uptake of the plant and an increase in the ratio of 'radioactive' to total phosphorus with time.

The fact that the easy exchangeability already found for lead, which is only incidentally present in plant tissues, has also been ascertained for phosphorus, one of the chief constituents of plants, indicates that we have to do with a general property of plant constitution. To investigate this point closer, it is intended to continue these experiments with other elements.

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91. EXCHANGE OF PHOSPHORUS ATOMS IN PLANTS AND SEEDS

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IN a letter to *Nature*⁽¹⁾ we communicated the results of experiments carried out on maize plants using radioactive phosphorus as an indicator of the behaviour of the phosphorus atoms. The results obtained showed unambiguously that the greater part of the phosphorus atoms present in the leaves and in the roots of the plants investigated is not permanently in one place, but migrates from leaf to leaf. We extended the experiments described above and investigated the behaviour of phosphorus in the sunflower.

The plant was first grown in an ordinary nutritive solution until a set of leaves, which we will call 'lower leaves', appeared. We then placed the plant in a second nutritive solution in which the phosphorus atoms were replaced by 'labelled' phosphorus atoms (mixed with radioactive phosphorus atoms). The second set of leaves, which we will denote as 'upper leaves', were grown in this solution and contained accordingly labelled ('radioactive') phosphorus atoms. For the sake of simplicity we will assume that the 'lower' leaves did not grow further in the second solution but retained their original size; then we must distinguish between two extreme cases: (*a*) the phosphorus atoms do not migrate;

TABLE 1

Time in days	Part of the plant	Total P (mgm)	Labelled P (per cent)	Unlabelled P (mgm)
	Lower leaf	0.980	0	0.980
	Upper leaf			
	Stem			
4	Lower leaf	0.848	43.6	0.478
	Upper leaf	0.840	51.5	0.407
	Stem	0.361	63.7	0.131
9	Lower leaf	0.742	66.9	0.246
	Upper leaf	2.838	65.6	0.976
	Stem	0.844	85.6	0.122

(*b*) the phosphorus atoms migrate. In case (*a*) labelled phosphorus atoms should only be found in the upper leaves; in case (*b*) the labelled phosphorus atoms should be equally distributed between the upper and lower leaves.

As is seen from Table 1, the lower leaves contain an appreciable amount of labelled phosphorus atoms, clearly showing that in the case of the sunflower, just as in that of the maize plant, a very considerable migration of the phosphorus atoms takes place, and the probability of a labelled phosphorus atom being found in a preformed leaf is not very

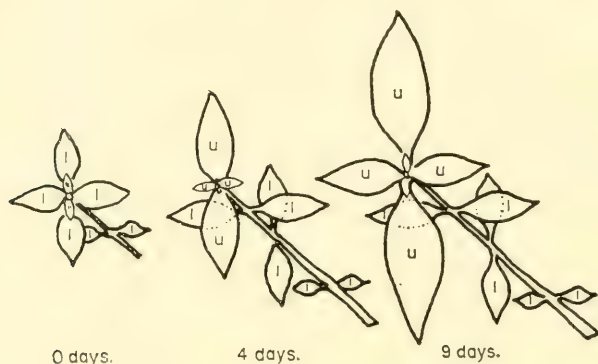


Fig 1.

different from the probability of its being found in a leaf grown in a labelled solution. In comparing the figure obtained for the lower and upper leaves, we have to take into account the fact that the lower leaves increase in size to some extent while the plant is in the labelled solution. However, this increase was found to be very slight, as to be seen from the third vertical column of Table 1 and also from Fig. 1, which illustrates the plant at the three different stages of its growth.

From the above, it follows that the greater part of the phosphorus atoms are not fixed in position in the leaves but can migrate. The bulk of the phosphorus atoms found in plants are presumably present as inorganic phosphate, and carried about with the circulation stream in the plant. To what extent the phosphorus atoms present in organic combination are permanently localized is under investigation.

In connexion with the experiments discussed above, we tried to ascertain if any exchange of phosphorus atoms takes place between an isolated leaf and a nutritive solution. These experiments were carried out with the leaves of a sunflower grown in labelled nutritive solutions. Some of the leaves were ignited and analysed at once after separating them from the stem, while other leaves were placed for 48 hours in a nutritive solution containing unlabelled phosphorus to give a chance

for exchange to occur between the labelled phosphorus in the leaf and the normal phosphorus in the solution. No labelled phosphorus could, however, be found in the nutritive solution.

In another set of experiments we placed cut leaves of sunflowers, grown in a normal nutritive solution, in labelled solution for 48 hours; in all the cases investigated an uptake of a few per cent of the labelled

TABLE 2. — MAIZE

No. of days	Part of the plant	Total P (mgm)	Labelled P (mgm)
3.9	Rootlet + leaf + scutellum ...	0.511	0.0134
	Endosperm	0.098	0.0001
7.0	Rootlet + leaf + scutellum ...	0.778	0.0275
	Endosperm	0.068	0
13.8	Rootlet + leaf + scutellum ...	1.008	0.0576
	Endosperm	0.016	0

TABLE 3. — PEA

No. of days	Part of the plant	Total P (mgm)	Labelled P (mgm)
3.9	Rootlet + leaf	0.167	0.0118
	Cotyledon	0.281	0.0013
7.0	Rootlet + leaf	0.445	0.0411
	Cotyledon	0.314	0.0033
13.8	Rootlet + leaf	0.481	0.0508
	Cotyledon	0.151	0.0050

phosphorus atoms into the cut leaf was found. As was to be expected, a cut leaf takes up not only the solvent but also the solute from the solution in which it has been immersed.

Finally, we would like to mention some experiments carried out with seeds. Two different series of experiments were carried out, one with maize seeds, the other with those of the pea. In both cases the seeds were germinated until rootlets 2—3 cm long were formed. Then the seeds were placed in small flasks with the rootlets dipping into a nutritive solution containing labelled phosphorus. In the case of the maize seeds (Table 2) the germ and endosperm were removed after the lapse of 4—14 days and analysed separately. While the germ was found to contain an appreciable amount of labelled phosphorus taken up from the nutritive solution, the endosperm did not contain the slightest trace; this shows that no exchange takes place between the phosphorus atoms of the germ and

the endosperm. In the case of the pea, there is no such marked distinction between germ and endosperm, the cotyledons occupying most of the space inside the seed, so that the labelled phosphorus atoms are taken up by the different parts of the seed. The concentration of the labelled phosphorus in the leaves and rootlets was here considerably higher than in the rest of the seed (cotyledons), as is seen from Table 3.

Reference

- I. G. HEVESY, K. LINDERSTRØM-LANG and C. OLSEN, *Nature* **137**, 66 (1936).

92. INTERACTION BETWEEN THE PHOSPHORUS ATOMS OF THE WHEAT SEEDLING AND THE NUTRIENT SOLUTION

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THE animal organism is bound to take up appreciable amounts of mineral constituents with its daily food; correspondingly, appreciable amounts of such constituents are daily excreted by the organism. In contradistinction to the animal body, the uptake of mineral constituents by the plant is not necessarily followed by a loss of such constituents. The application of isotopic indicators, however, has shown that ions taken up by the plant can be removed under the action of other ions present in the soil or in the nutrient solution. At an early date⁽¹⁾, it was shown that minute amounts of lead labelled by the admixture of the lead isotope thorium *B* taken up by the roots of *Vicia faba* to a large extent can be removed by an excess of non-labelled lead added to the nutrient solution. Most other ions were found, as shown in Table 1, to be much less effective in removing the labelled lead ions.

The discovery of artificially radioactive isotopes made it possible to study the removal of essential constituents from the plant organism as,

TABLE 1. — REMOVAL OF MINUTE AMOUNTS OF LABELLED LEAD FROM THE ROOTS OF *Vicia faba* WHEN IMMERSSED IN A 10^{-2} N SOLUTION OF DIFFERENT SALTS

Solution used	Per cent of the lead initially present in the root, which remained after treatment
Lead nitrate (inactive)	5
Cupric nitrate	3
Cadmium nitrate	34
Zinc nitrate	38
Chromium nitrate	43
Barium nitrate	74
Sodium nitrate	76

⁽¹⁾ G. HEVESY, *Biochem. J.* **17**, 439, 1923.

for example, that of potassium. MULLINS and BROOKS⁽¹⁾ placed cells of *Nitella coronata* first in a solution containing radioactive potassium and, later, in solutions of different chlorides. The curves for the loss of radioactive ions from *Nitella* were found to be exponential and following the equation.

$$C_p = C_0 e^{-kt}$$

TABLE 2. — REPLACEMENT OF
POTASSIUM IONS PRESENT IN THE
PROTOPLASM OF *Nitella* BY OTHER IONS

Replacing ion	Value of k
Li	0.12
Na	0.16
K	0.83
Rb	2.00
HOH	0.05

where C_p is the concentration of radioactive ions in the protoplasm, C_0 the initial concentration of ions in the protoplasm, k a constant giving the slope of the logarithmic curve, and t the time of immersion of the cell in the solution of inactive ions. The values of k which were found are given in Table 2; they represent the rate of loss of ions from the cell. As seen in the table, the loss of labelled potassium is greatest in the solution containing non-labelled potassium or rubidium and is minimal in pure water. Sodium and lithium are much less effective in replacing the labelled potassium than is potassium.

A similar result was thus obtained as in the case of lead. It was furthermore shown by JENNY and OVERSTREET⁽²⁾ and BROYER and OVERSTREET⁽³⁾ that ionic exchange movements may take place during periods of and under conditions favourable to active solute uptake.

JENNY, OVERSTREET and AYERS,⁽⁴⁾ who studied the contact interchange of cations between plant roots and soil colloids, found that barley plants containing radioactive potassium retain this isotope against distilled water, but release it to salt solutions and clay suspensions.

HEVESY, LINDERSTRØM-LANG and OLSEN⁽⁵⁾ placed sunflower and maize seedlings in culture solutions containing radio-phosphorus. After

⁽¹⁾ L. J. MULLINS and S. C. BROOKS, *Science* **90**, 256 (1939); comp. also S. C. BROOKS, *Trans. Faraday Soc.* **33**, 1002 (1937); *Proc. Soc. Exp. Med. Biol.* **38**, 856 (1938).

⁽²⁾ H. JENNY and R. OVERSTREET, *J. Phys. Chem.* **43**, 1185 (1939).

⁽³⁾ T. C. BROYER and R. OVERSTREET, *Amer. J. Bot.* **27**, 425 (1940).

⁽⁴⁾ H. JENNY, R. OVERSTREET and A. D. AYERS, *Soil Sci.* **48**, 9 (1939).

⁽⁵⁾ G. HEVESY, K. LINDERSTRØM-LANG and C. OLSEN, *Nature* **137**, 66 (1936); **139**, 149 (1936).

the lapse of some days, they found that the radio-phosphorus taken up by the plant was almost uniformly distributed between all phosphorus present in the plant. The migration of radio-phosphorus in the plant was also demonstrated by BIDDULPH⁽¹⁾. When placing wheat seedlings containing radio-phosphorus in an inactive culture solution, the radio-phosphorus content of the leaf was found by BREWER and BRANLEY⁽²⁾ to have decreased only slightly with time, while the radioactivity of the solution increased slowly through loss of phosphorus from the roots and lower portions of the stalk.

The author was interested in determining to what extent the uptake of phosphate ions by growing plants is followed by a simultaneous loss of phosphate by the plant. To this purpose he studied the uptake and the release of labelled phosphate ions by wheat seedlings.

EXPERIMENTAL

The most direct way to attack the problem outlined above is the growing of seedlings in a nutrient solution containing labelled phosphate and, after the roots are carefully washed, the replacement of the radioactive solution by a non-active one. An exudation of labelled phosphate from the plant should lead to an accumulation of radioactive phosphorus in the originally inactive solution. Against this procedure the objection may be raised that the roots of a plant growing in a solution containing labelled phosphate may be covered by active iron phosphate or another sparingly soluble phosphate, and the activity observed in the originally inactive solution may originate from this deposit and not from the plant proper. Therefore, the author made use of the technique of parted roots.

Wheat grains are placed for speering on the edge of a glass cuvette which is placed in a beaker. By making use of this technique seedlings with parted roots are obtained. A fortnight old the seedlings are placed in an apparatus composed of two concentric cylinders. One part of the roots is placed in the inner, the other part in the outer cylinder. The diameter of the inner cylinder is 2.4 cm, that of the outer cylinder 3.8 cm. Their length is 20 cm. Both cylinders are filled to a height of 18 cm with nutrient solution of the following composition per liter.⁽³⁾

0.055	gm	KH_2PO_4
0.10	gm	$\text{Mg}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$
0.35	gm	KNO_3
0.10	gm	ferric citrate
0.5	mgm	$\text{MnSO}_4 \cdot 4 \text{H}_2\text{O}$
0.25	mgm	$\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$
0.25	mgm	H_3BO_3

Sodium hydroxide is added until pH — 5.8 is reached. After the lapse of about 1 week, for example, labelled phosphate of negligible weight and having an

⁽¹⁾ O. BIDDULPH, *Science* **89**, 393 (1939).

⁽²⁾ A. K. BREWER and A. BRANLEY, *Science* **91**, 269 (1940).

⁽³⁾ L. OLSEN, L. R. des Travaux du Lab. Carlsberg, **21**, 17 (1935).

activity of 1 to 10 μ Curie is added to the solution of the inner cylinder. The uppermost 2 cm of the cylinders do not contain liquid, furthermore, the walls of this part of the cylinders are coated with a layer of paraffin. These measures are taken in order to avoid the creeping of salt along the surface of the cylinder wall from the active into the inactive solution. The absence of such a disturbing effect was ascertained by adding dyes to the inner solution. No passage of dye could be observed. Furthermore, as discussed on p. 896, the amount of inactive phosphate present in the outer cylinder has a marked influence on the amount of radioactive phosphate which migrates from the inner into the outer cylinder. This finding can only be explained by a passage of the labelled phosphorus from the inner solution through the roots into the outer solution, as a creeping of a salt along the surface of the roots can hardly be influenced by small changes in the salt content of the liquid into which the creeping salt ultimately finds its way.

To investigate the activity which accumulated in the outer cylinder the liquid is collected through an inverted *U* tube, slightly acidified with hydrochloride acid and filtered to remove solid particles possibly present. 70 mgm Na_2HPO_4 are then added and the phosphorus is precipitated as ammonium magnesium phosphate. This precipitate contains the labelled phosphate which had migrated through the "inactive" roots into the outer solution. We denote the roots placed in the inner solution and, thus, growing in a radioactive medium as "active" roots. At the end of the experiment, we extract the acid-soluble phosphorus of the "inactive" roots with 5 p. c. cold trichloroacetic acid. A part of this solution is used for colorimetric determination of its "free" *P* content, while another part is taken to determine the radioactivity of the "free" *P*. After the addition of 70 mgm Na_2HPO_4 , the phosphorus content of the solution is precipitated as ammonium magnesium phosphate and is placed under the Geiger counter tube. Another aliquot of the solution is digested and the specific activity of the total acid-soluble *P* is measured. In another experiment the plant is digested *in toto* and its *P* content and its radioactivity are determined as well.

RESULTS

The distribution of the total *P* in the different parts of the plant and the radioactive *P* (^{32}P) content of the active and inactive roots is shown in Tables 3 and 4. In some of the seedlings investigated after the lapse of

TABLE 3. — DISTRIBUTION OF THE TOTAL *P* IN THE DIFFERENT PARTS OF THE PLANT AND THE ^{32}P CONTENT OF THE ROOTS

Plant	Active roots		Inactive roots		Leaves and stem	
	Dry weight in mgm	Total <i>P</i> in mgm	Dry weight in mgm	Total <i>P</i> in mgm	Dry weight in mgm	Total <i>P</i> in mgm
1	23.2	0.825	18.8	0.748	254.0	2.50
2	28.8	0.970	22.5	0.825	323.6	3.05
3	21.2	0.825	17.5	0.804	258.8	2.22
4	20.2	0.432	19.6	0.500	234.8	2.40
5	20.1	0.540	18.2	0.520	218.2	2.50
Average value	22.7	0.718	19.3	0.680	257.8	2.53

14 days the ^{32}P content of the "inactive" roots amounts to about $1/30$ of the ^{32}P content of the "active" roots. This result indicates a fairly slow passage of the ^{32}P from the "active" into the "inactive" roots. The activity values can clearly not become equal even after a very long time, as the "inactive" roots are placed in an inactive solution which is daily replaced by another inactive solution.

PERCENTAGE OF ^{32}P PRESENT IN THE ROOTS
14 DAYS AFTER ADDITION OF LABELLED
PHOSPHATE TO THE INNER SOLUTION

Plant	Active roots	Inactive roots
1	9.8	0.31
2	11.1	0.41
3	12.1	0.34
4	11.4	0.32
5	14.8	0.58
Average value ..	11.8	0.39

The dry weight and the total phosphorus content of the active and the inactive roots do not much differ, while the ^{32}P content of the "inactive" roots makes out only $1/30$ of the ^{32}P content of the "active" roots.

The percentage of labelled phosphate migrating from the active solution through the plant into the inactive solution in the course of 48 hours is seen in Table 4. Six weeks old seedlings were used, the active phosphate being added 14 days before the experiment was carried out.

TABLE 4. — PERCENTAGE OF
ACTIVE PHOSPHATE MIGRAT-
ING IN THE COURSE OF 1 DAY
FROM THE ACTIVE INTO THE
INACTIVE SOLUTION

Plant	Percentage migrated
1	0.051
2	0.049
3	0.058
4	0.039
5	0.031
6	0.030
7	0.034
8	0.052
9	0.031
10	0.057
11	0.044
12	0.039

While the above figures show that an easily measurable percentage of the radioactive phosphate ions and, correspondingly, of all phosphate ions added to the solution contained in the inner cylinder passed through the plant into the outer solution, it is difficult to estimate the quantitative significance of these figures. During their passage through the plant the active phosphate ions get more and more "diluted" by non-active phosphate ions present in the plant. By this dilution process the sensitivity of the isotopic indicator much increases. Let us assume that we add 10^6 radioactive units to the inner solution which contains 1 mgm phosphorus; the presence of 1 radioactive unit in the outer liquid will then not indicate the exodus of $1 \cdot 10^{-6}$ mgm from the "inactive" roots into the outer solution, only, but a much larger amount of phosphate in view of the above described increase in sensitivity of the radioactive indicator during the passage of the ^{32}P atoms through the plant.

To determine the sensitivity of the radioactive indicator the following procedure is applied. The labelled phosphate found in the outer solution can be assumed to have migrated mainly as free phosphate from the roots into the solution (cf. p. 903); therefore, we isolate the free phosphate from the inactive roots at the end of the experiment and determine its specific activity. Let us assume the specific activity to be 10,000 activity units per mgm P. In this case, the presence of 1 activity unit in the "inactive" outer solution indicates the exodus of 10^{-4} mgm from the "inactive" roots into the inactive solution.

Amount of phosphorus given off by the roots

The results of experiments in which the procedure described above is applied are to be seen in Tables 5, 6, and 7.

TABLE 5. — AMOUNT OF PHOSPHORUS MIGRATING FROM THE "INACTIVE" ROOTS INTO THE OUTER (INACTIVE) NUTRIENT SOLUTION IN THE COURSE OF 1 DAY. AVERAGE TEMPERATURE 20°C

Free P content of		Percentage of ^{32}P added present in the free P of	
active roots	inactive roots	active roots	inactive roots
67.5 γ	150 γ	4.02	0.84

To 1 γ of free P present in the inactive roots thus corresponds 0.0056 p. c. of the ^{32}P added to the inner solution. After the lapse of one day, 0.037 p. c. of the ^{32}P added was found to be present in the "inactive" solution. Consequently, in the course of one day 6.6 γ of P originally present in the roots migrated into the culture solution.

After the lapse of 46 days, 53.9 p. c. of the ^{32}P added were found to be taken up by the plant. The volume of the active and inactive solutions amounted to 70 and 60 cm^3 , respectively, and the P content at the start of the experiment to 1.13 and 0.7 mgm, respectively. As the inactive solution was daily renewed, the P content of this solution was at the end of the experiment almost the same as at the start of the experiment, while the P content of the active solution amounted to only 46 p. c. of its initial value.

TABLE 6. — AMOUNT OF PHOSPHORUS MIGRATING FROM THE “INACTIVE” ROOTS INTO THE OUTER (“INACTIVE”) NUTRIENT IN THE COURSE OF 1 DAY

Free P content of		Percentage of ^{32}P added present in the free P of	
active roots	inactive roots	active roots	inactive roots
67.5 γ	42.3 γ	2.32	0.265

To 1 γ of free P present in the inactive roots thus corresponds 0.0063 p. c. of the ^{32}P added to the inner solution. After the lapse of one day, 0.076 p. c. of the ^{32}P added was found to be present in the “inactive” solution, thus in the course of one day 12 γ originally present in the roots migrated into the culture solution. After the lapse of 38 days, 78 p. c. of the ^{32}P added were found in the residual parts of the plant which had a P content of 726 γ .

TABLE 7. — AMOUNT OF PHOSPHORUS MIGRATING FROM THE “INACTIVE” ROOTS INTO THE OUTER (“INACTIVE”) NUTRIENT SOLUTION IN THE COURSE OF 1 DAY

Free P content of		Percentage of ^{32}P added present in the free P of	
active roots	inactive roots	active roots	inactive roots
Plant			
1 109 γ	158 γ	15.9	0.63
2 173	364	12.7	1.56
3 150	145	6.2	0.41

Ad 1. To 1 γ free P present in the active roots corresponds 0.0040 p. c. of the ^{32}P added. In the course of one day, 0.04 p. c. of the P added migrated into the inactive solution, thus in the course of 1 day 11 γ originally present in the roots moved into the culture solution.

- Ad 2. To 1 γ free P present in the inactive roots corresponds 0.0043 p. c. of the ^{32}P added. In the course of one day, 0.068 p. c. of the P was found to have migrated into the inactive solution, thus in the course of 1 day, 15.7 γ originally present in the roots moved into the culture solution.
- Ad 3. To 1 γ free P present in the inactive roots corresponds 0.0028 p. c. of the ^{32}P added. In the course of 1 day, 0.049 p. c. of the P added was found to have migrated into the inactive solution, thus in the course of 1 day, 17.5 γ originally present in the roots moved into the culture solution.

The following distribution of the ^{32}P added to three weeks old wheat plants was found to take place 4 weeks after the start of the experiment.

TABLE 8

Plant	Active roots %	Inactive roots %	Residual part of the plant %	Nutrient solu- tion %
1	20.2	2.4	70.7	6.7
2	17.0	4.9	65.8	12.3
3	22.3	3.3	73.2	1.2

The amount of phosphorus migrating from the roots of different plants into the nutrient solution in the course of one day is seen in Table 9.

TABLE 9. — AMOUNT OF PHOSPHORUS
MIGRATING FROM THE ROOTS OF DIFFE-
RENT PLANTS INTO THE NUTRIENT SOLU-
TION IN THE COURSE OF ONE DAY

6.6 γ
12.0 γ
11.0 γ
15.7 γ
17.5 γ
Average value 12.6 γ

Amount of phosphorus taken up by the roots

To determine the amount of phosphorus taken up by the roots of plants of about the same size as those used in the experiments described, we measured the percentage of the ^{32}P added to the inner solution which accumulated in the plant in the course of 1 day. From these figures and the phosphorus content of the solution the amount of phosphorus taken up by the roots can be calculated. The results obtained are seen in Table 10.

TABLE 10. — PASSAGE OF PHOSPHORUS FROM THE SOLUTION THROUGH THE ROOTS INTO THE PLANT IN THE COURSE OF 1 DAY. AVERAGE TEMPERATURE 22° C

Plant	³² P content in counter units in			P content of inner solution	Percentage ³² P uptake by the plant	Phosphorus taken up by the plant
	solution	roots	residual part			
1	33 700	1 830	1 950	630 γ	10.2	64 γ
2	38 300	3 260	1 680	622 γ	11.1	69 γ
3	43 500	3 720	1 830	675 γ	11.3	76 γ
4	36 600	2 230	1 770	710 γ	9.8	70 γ
5	31 500	1 920	1 510	702 γ	9.9	69 γ
Average value				676 γ	10.5%	68 γ

Thus, 68 γ passed through the roots in upward movement during 1 day. As we found under the same conditions 12.6 γ of P to have moved from the roots into the solution, we have to conclude that for about 6 phosphorus atoms taken up by the roots 1 phosphorus atom moves in opposite direction, viz. from the roots into the nutrient solution. This result was found from a nutrient solution containing 0.4 millimol. phosphorus per liter. By increasing the phosphate concentration of the "inactive" solution, the passage of phosphate from the roots into the solution increases; when decreasing the phosphate concentration the passage decreases, as seen in Table 11. The latter result we should expect to obtain for an interchange process in contrast to a dissolution process. In the case of an interchange process, the probability of the removal of an active P atom by an inactive one increases when the concentration of the inactive P atoms is increased.

TABLE 11. — REMOVAL OF LABELLED PHOSPHATE FROM THE ROOTS OF WHEAT SEEDLINGS IN NUTRIENT SOLUTIONS OF DIFFERENT PHOSPHORUS CONTENTS IN THE COURSE OF 1 HOUR

Ratio of the removal of labelled phosphate from the roots by a nutrient solution containing	
4 and 0.16 millimol. P per liter, respectively	0.4 and 0 millimol. P per liter, respectively
2.5	4.4
1.4	2.1
3.2	5.3
1.4	5.6
2.4	2.2
2.2	2.7
Average value 2.2	4.7



In an interpretation of the figures obtained from a solution free of phosphorus it should be kept in mind that not only inactive P interchanges with active P, but also other anions present in the nutrient solution, though these can be expected to be less effective in removing active P from the roots than is inactive P (cf. p. 892). Furthermore a migration of anions simultaneously with an equivalent amount of cations from the roots into the nutrient solution must also be envisaged.

Another series of experiments gave instead of 2.2 the average value of 3.0 for the ratios listed in Table 11.

A possible explanation of the fact that an increase in the phosphate concentration of the nutrient solution leads to an increased migration of ^{32}P from the roots is the following. An increased non-labelled phosphate concentration of the nutrient solution leads to an increased non-labelled phosphate concentration of the liquid phase of the roots. The non-labelled phosphate present in the liquid phase of the roots gets into a kinetic, respectively enzymic, exchange-equilibrium with a part of the labelled free phosphate and the labelled phosphate incorporated in organic compounds, respectively, present in the solid phase of the roots. The replacement of the labelled phosphate of the roots by non-labelled phosphate is promoted by an increase in the non-labelled phosphate concentration of the liquid phase which, in turn, leads to an enhanced exodus of labelled phosphate from the roots into the nutrient solution.

In distilled water, we would expect the roots to lose less radio-phosphate than in a phosphorus free nutrient solution. As seen from Table 12, the loss of radio-phosphate in distilled water differs from our expectance.

TABLE 12. — RATIO OF REMOVAL OF
LABELLED PHOSPHATE FROM THE ROOTS
OF WHEAT SEEDLINGS IN DISTILLED
WATER AND IN PHOSPHATE-FREE
NUTRIENT SOLUTION RESPECTIVELY

2.1	1.5
3.6	2.3
3.4	5.7
2.4	5.8
2.5	1.3
Average value 3.1	

According to Table 12, the roots lose more ^{32}P in distilled water than in a phosphorus-free nutrient solution. In another series of experiments, the average value of the above-mentioned ratio was found to be 3.4. The exudation of nucleotides containing radio-phosphorus is, as discussed on p. 903, much smaller than the loss of phosphate and, correspondingly, a precipitation of some of the nucleotides together with the precipitation

of magnesium ammonium phosphate cannot be made responsible for the result obtained.

The distilled water was redistilled from a glass vessel and thus was free from traces of heavy metals, the presence of which can thus not be made responsible for the behaviour of the roots. The result obtained suggests therefore that distilled water already in experiments taking 1 hour only influences the permeability of the root cells. The distilled water, while influencing the phosphate permeability of the root cells, may not necessarily produce an injury of the cells. *Nitella* cells kept in distilled water for 3 days, for example, were found to lose their irritability and their characteristic behaviour with potassium, but no sign of injury was observed and such cells were found to live indefinitely when transferred to pond water⁽¹⁾.

Exudation of phosphorus compounds by the roots

In our considerations we started from the assumption that labelled phosphate found in the "inactive" nutrient solution containing phosphate is due mainly to an interchange between the phosphate ions present in the plant and those present in the nutrient solution. This assumption is not quite correct, as LUNDEGÅRDH⁽²⁾ and his collaborators have shown that living roots always exude small amounts of organic substances. These authors observed that the said substances contained phosphorus. LUNDEGÅRDH and STENLID⁽³⁾ experimented with roots of young seedlings, 2—4 days old. The roots were cut off from the seeds and submersed in aerated distilled water or aerated dilute solutions of salts or mineral acids. Most of their experiments were carried out in distilled water only. They found that 8 per cent of the exudate of wheat roots had been composed of nucleotides, the amount being determined both spectrochemically and by making use of the benzidine test. The amount of nucleotides exuded in the course of 5 hours was estimated by them to correspond to 0.5—1 per cent of the total dry weight of the roots (the tips found to exude 2—4%). The decrease in exudation after 5 hours was interpreted as being probably due to a cessation of growth of the seedlings after that time.

In view of these results, we measured the amount of labelled nucleic acid exuded in our experiments and determined the percentage of ³²P migrated from the roots into the nutrient solution which could be

(1) S. E. HILL, *Proc. Soc. Exp. Med.* **32**, 413 (1934 · 1935).

(2) H. LUNDEGÅRDH, *Die Nahrungsaufnahme der Pflanze*, Jena (1932); H. LUNDEGÅRDH, H. BURSTRÖM and E. RENNFELT, *Svensk Bot. Tidsskr.* **26**, 271 (1932); H. BURSTRÖM, *l.c.* **23**, 157 (1934).

(3) H. LUNDEGÅRDH and G. STENLID, *Kungl. Svenska Vetensk. Akad. Ark. f. Bot.* **31** A, No. 10 (1944).

ascribed to nucleic acid phosphorus. The experiments were carried out with seedlings grown in a labelled nutrient solution. The specific activity of the total P being in such a case identical with the specific activity of the nucleic acid P, we can calculate from the specific activity of the nucleic acid exuded into the solution in which the seedling is placed, and from the specific activity of the total P of the seedling (cf. p. 903) the amount of nucleic acid exuded. The application of the technique of parted roots is clearly not advisable when determining the amount of labelled nucleic acid exuded into the nutrient solution or distilled water.

As, simultaneously with the exudation of labelled nucleic acid into the nutrient solution, a passage of labelled phosphate takes place, it is necessary to purify the radioactive nucleic acid from the radioactive-free phosphate. This was obtained in the following way. To an aliquot (25 cm³) of distilled water, in which a wheat seedling was placed for 1 hour, we added 100 mgm of inactive ribonucleic acid, simultaneously adding 0.2 cm³ 1 N NaOH. Then, for each cm³ solution, 0.44 cm³ saturated NaCl, 0.025 cm³ 1/2-mol. Na₂HPO₄, and 0.087 cm³ 1/2-mol. CaCl₂ were added. The amounts of the ingredients to be used were kindly suggested to the author by Professor EINAR HAMMERSTEN. Subsequently, the calcium phosphate precipitate was removed by centrifuging the solution. The liquid obtained was poured into 5 times its weight of glacial acid. 16.4 mgm were secured, showing an activity of 2.8 activity units or 0.17 units per mgm.

Control experiments described below made it clear that such a procedure is effective in removing active phosphate from the nucleic acid; the same was the case when the nucleic acid precipitate was redissolved and reprecipitated by an alcoholic solution of hydrochloric acid. From the fact that, within the errors of experiment, the activity of the nucleic acid fraction did not change when it was reprecipitated we can conclude that it did not contain adhering active phosphate. The activity of 1 mgm nucleic acid was found to be 1.7 and 1.6 activity units, respectively, before and after reprecipitation.

The control experiments were carried out in the following way. 50 mgm inactive ribonucleic acid was dissolved, 250,000 activity units were added and NaOH, NaCl, Na₂HPO₄ and CaCl₂ were added as well. The activity of the 5.3 mgm nucleic acid secured as described above was less than 10⁻⁶ part of the activity added, showing that the separation of free phosphate from the nucleic acid was very effective.

We may expect the active nucleic acid present in the solution and the inactive nucleic acid added to follow each other during the separation process and, as we recovered only 16.4 p. c. of the inactive nucleic acid added (100 mgm) in the experiment described above, we can expect a corresponding fraction of the active nucleic acid to have been recovered as well. We have thus to multiply the 2.8 activity units with 6.1 to

find the total activity of nucleic acid present in 25 cm³ solution. When carrying out this calculation we assume the weight of the active nucleic acid to be negligible compared with the weight of the 100 mgm inactive nucleic acid added.

As we, furthermore, analysed only a $\frac{25}{189}$ part of the solution, we have to multiply the result obtained by $\frac{189}{25}$. The total activity of the nucleic acid exuded works out to be 129, while a simultaneous analysis of the free phosphate present in the solution indicates a total activity of 716. Thus, 15 per cent of the ³²P which migrated from the roots into the distilled water originate from the ³²P content of nucleic acid exuded. The ratio of free phosphate activity and nucleic acid activity found to be present in the distilled water in which the roots had been placed for 1 hour in 3 experiments is seen in Table 14.

TABLE 14. — ACTIVITY OF THE PHOSPHATE FRACTION AND THE NUCLEIC ACID FRACTION PRESENT IN THE DISTILLED WATER IN WHICH WHEAT SEEDLING ROOTS HAD BEEN PLACED FOR 1 HOUR

Phosphate	Nucleic acid	Ratio
716.0	129	5.6
3 295	413	8.0
10 620	1 300	8.1

Amount of nucleic acid exuded

The wheat seedlings used in our experiments were grown almost from the start in labelled solution. The activity of 1 mgm of seedling P can therefore be assumed to be equal to the activity of 1 mgm of exuded nucleic acid phosphorus. In the first set of experiments, the total activity of the nucleic acid P exuded varied between 0.04 and 0.09 per cent of the total activity of the seedling. The phosphorus content of the dry roots of the seedling (3%) is only $\frac{1}{3}$ of the phosphorus content of the nucleic acid; thus, the amount of nucleic acid exuded in the course of 1 hour varies between 0.013 and 0.03 per cent of the dry weight of the roots. LUNDEGÅRDH and STENLID have found an exudation percentage of 0.5 to 1 per cent in the course of 5 hours or, assuming the amount exuded in the course of 1 hour to be $\frac{1}{5}$ of the amount exuded in the course of 5 hours, 0.1 to 0.2 per cent per hour. Our values amount to only about $\frac{1}{8}$ of the amount observed by LUNDEGÅRDH and STENLID. This difference is, however, to be expected in view of these authors' account. Our experiments were carried out on 2 to 3 months old plants, while LUNDEGÅRDH and STENLID per-

formed their experiments on roots of young seedlings, viz. 2—4 days old. According to these experimentors, the process of exudation of nucleotides from the root is a characteristic feature of the still growing parts; the lower 5—10 mm of the roots exude per unit dry weight 3—4 times as much nucleotide as roots of 30—40 mm length. The nucleotides are assumed to participate in the building up of cellulose fibres which constitute the cell wall. In the course of development of the cells, the nucleotides are gradually liberated and the exuded molecules are not renewed in ageing cells. The frequency of nucleotides diminishes consequently and the growth slackens. Thus, the exudation of nucleotides can be expected to diminish with increasing age of the seedlings.

Summary

Wheat seedlings with parted roots are grown. One part of the roots is placed in an inactive nutrient solution, the other part in a nutrient solution containing labelled phosphate. The radio-phosphorus is found to migrate through the plant from the active into the inactive nutrient solution.

When the nutrient solution contains 0.4 millimol. P per liter, for 6 phosphate atoms taken up by the plant about 1 is found to migrate from the roots into the nutrient solution.

An increase in the phosphate concentration of the nutrient solution was found to increase the amount of labelled phosphate given off by the roots, and vice versa. Such a result is to be expected if we are mainly faced with an interchange between the phosphate ions of the roots and the phosphate ions of the nutrient solution.

Roots placed in distilled water for 1 hour give off more labelled phosphate than roots placed in a nutrient solution free from P. Distilled water influences thus the root cells of wheat seedlings.

Beside labelled phosphate, roots of wheat seedlings give off a minor amount of labelled nucleotides when placed in distilled water. This observation supports the results obtained by LUNDEGÅRDH and his collaborators.

COMMENT ON PAPERS 89—92

THE first application of radioactive tracers in life-sciences was the investigation of the uptake of lead by plants using ThB as an indicator of lead (paper 89, published in 1923). The first application of an artificially produced radioactive isotope, that of ^{32}P , in botanical studies was the investigation with LINDERSTRØM-LANG and OLSEN of the uptake of ^{32}P by the maize plants (paper 90, published in 1936) and by the sunflower and germinated maize and pea seeds (paper 91). The extrusion of phosphorus compounds from the roots of labelled plants was studied later (paper 92). These modest beginnings were followed by a spectacular development in the field of Botany, Agriculture and Forestry. The present position and future trends of this development are stated among others by the European Productivity Agency Of The Organisation For European Economic Co-operation. Paris 1958 and 1959.

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93. EXCHANGE OF NITROGEN ATOMS IN THE LEAVES OF THE SUNFLOWER

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MAKING use of radioactive phosphorus as an indicator we found, in an earlier investigation, that the greater part of the phosphorus atoms present in the leaves and the roots of the plants investigated is not permanently on one place but migrates from leaf to leaf (HEVESY, LINDERSTRØM-LANG and OLSEN, 1936, 1937). We investigated later, on similar lines, the behaviour of nitrogen. The investigation of the behaviour of nitrogen seemed to be of great interest, since the greatest part of nitrogen is present in the plants in the form of protein compounds, and the replacement of such nitrogen atoms by others involves the breaking of nitrogen bonds and a restitution of protein molecules by enzymatic action.

Nitrogen has no known radioactive isotope of suitable life; however, the heavy nitrogen isotope ^{15}N can be used as an indicator. Through the great kindness of Professor UREY, we came into possession of large amounts of ammonium chloride, which contained ^{15}N in a very appreciable excess over that present in ordinary ammonium chloride. While the nitrogen of the air contains 0.37 per cent. ^{15}N , Professor UREY's preparation contained 2.5 per cent.

Sunflowers were grown in an ordinary culture solution containing normal ammonium sulphate until a set of leaves, the lower ones, appeared. We then placed the plant in a second culture solution in which the normal ammonium content was replaced by "heavy" ammonium. After the lapse of three weeks, we transformed the nitrogen of the plants into ammonia by KJELDAHL's method. The "lower" leaves, which have grown only to a minor extent during the second stage of the experiment, and the "upper" leaves, which were to a very large extent formed during the second stage of the experiment, were separately treated. The upper leaves having been developed in a culture solution containing ^{15}N were bound to contain a corresponding amount of the heavy nitrogen isotope. In the "lower" leaves, ^{15}N could, however, only enter in excess

of the ^{15}N found in all plants, if the nitrogen atoms were released from the molecules in which they were incorporated and replaced by other nitrogen atoms carried to the place of synthesis by the circulation stream. A comparison of the ^{15}N content of the nitrogen prepared from the upper and the lower leaves, which was most kindly made with the mass-spectrograph by Professor SCHOENHEIMER and his collaborators (RITTENBERG, KESTON, ROSEBURY and SCHOENHEIMER, 1939), lead to the result that the ^{15}N content of the total nitrogen prepared from the "old" leaves amounted to about nearly half of that present in the "new" leaves. Thus, a large part of the nitrogen atoms present in the "old" leaves was replaced by other nitrogen atoms during the second stage of the experiment⁽¹⁾.

In our later experiments we have separated the protein nitrogen from the non-protein nitrogen present in the leaves. Furthermore, we have compared the nitrogen exchange in three different kinds of leaves: those which have not grown further while in a culture solution containing ^{15}N , those which have grown to some extent and, finally, those which were formed during that period alone.

EXPERIMENTAL PROCEDURE

Sets of 4 sunflower plants were grown in jars containing 5 liters of a complete culture solution with a content of 28 mgm normal nitrogen as ammonium sulphate per liter. After the lapse of three weeks, the solution was replaced by another solution containing, instead of normal nitrogen, nitrogen with 2.5 per cent ^{15}N content. After the lapse of 6 days, this solution was renewed. After the lapse of a further 6 days, the experiment was broken off. The leaves were sorted into three groups: (a) old leaves, which did not grow after the plants were transferred into the culture solution containing heavy nitrogen; (b) young leaves developed solely after the transfer; (c) intermediary leaves which could not with certainty be classified in one of the former groups. That the old leaves did not grow further in the solution containing heavy nitrogen was ascertained by measuring their size before the transfer into the solution containing the heavy nitrogen and at the end of the experiment. The new leaves were not yet visible at the time of transfer. As to the intermediary leaves, the size of one of the youngest leaves was measured before and after the experiment. The size of this leaf was found to have increased by nearly one half of its original value. The increase in size in the case of most of the other intermediary leaves was much less.

The leaves were dried at room temperature *in vacuo* over sulphuric acid. They were, subsequently, ground in a ball mill, and the powder obtained extracted with ice-cooled 2 per cent sodium chloride solution containing phosphate buffer (pH = 6.8). The operations were carried out in a room kept at 0°. To the extract

⁽¹⁾ These results were communicated at the meeting of the Semaine internationale contre le Cancer, Paris, November 1938. (Comp. ACTA [Unio internationalis contra cancerum], Vol. IV, No. 1-2, p. 175, 1939). Since, contrary to our later experiments which are described in this paper, in our earlier plants some growth of the "old" leaves took place during the experiment, the ^{15}N content of the old leaves was only partly due to replacement of old protein molecules by new ones.

TABLE 1

Leaves	Fraction	Nitrogen content in mgm
Young	I	17.6
	II	74.6
	III	653.3
Intermediary	I	15.6
	II	80.2
	III	150.9
Old	I	15.2
	II	38.0
	III	30.3

trichloroacetic acid was added, the solution obtained containing 5 per cent of this substance. By this process, the proteins were precipitated. They were filtered off and washed with 5 per cent trichloroacetic acid. We will denote this precipitate as fraction I. The trichloroacetic filtrate was evaporated, supplying fraction II. The residue of the extracted leaves is denoted as fraction III. All three fractions were kjeldahlized, supplying three ammonia fractions which were neutralized with hydrochloric acid.

The total nitrogen content of these samples was determined as ammonium by titration. To obtain the ^{15}N content of the samples from the ammonia distillates obtained, nitrogen was prepared by the method described by RITTENBERG, KESTON, ROSEBURY and SCHOENHEIMER. The ^{15}N content of these samples was determined with the mass-spectrograph.

TABLE 2

Leaves		^{15}N content <i>atom per cent excess</i>	Percentage of old nitrogen atom replaced by new ones, assuming the absence of growth during the experiment
Protein nitrogen	New	0.859	—
	Intermediary	0.654	76
	Old	0.106	12.3
Non-protein nitrogen ¹ soluble in NaCl solution	New	0.894	—
	Intermediary	0.703	79
	Old	0.114	12.7
Non-extractable nitrogen	New	0.939—0.936	—
	Intermediary	0.690	74
	Old	0.164	17.5

¹ Containing presumably also nitrogen split off from proteins during drying of the leaves.

The dry weight of the young leaves investigated amounted to 17.4 gm, that of the old leaves to 11.4 gm. The nitrogen content of the different fractions is seen in Table 1.

The dry substance of the three different types of leaves was found to contain 4.28, 1.80, and 0.73 per cent nitrogen, respectively.

The result of the mass-spectrographic investigation is seen in Table 2.

CONCLUSIONS

As already mentioned, the "old" leaves did not grow further while kept in the culture solution containing heavy nitrogen. The protein ^{15}N content of these leaves is, thus, due solely to an exchange process, involving the enzymatic splitting off of the nitrogen content of a part of the protein molecules present and their replacement by an enzymatic restitution with incorporation of ^{15}N atoms. As seen in Table 2, the extent of replacement amounted, in the course of 8 days, to 12.3 per cent of the total protein nitrogen present in the old leaves. We arrived at this figure by comparing the ^{15}N excess of the old leaves with that of the new leaves under the assumption that the new leaves were exclusively formed while the plant was kept in the culture solution containing heavy nitrogen. As to the intermediary leaves, the assumption that these leaves were exclusively grown in the last 12 days of the experiment is certainly not justified and the corresponding figure, quoted in the last column of Table 2, represents therefore an upper limit to the replacement. As already mentioned on p. 906 the growth of these leaves amounted certainly to less than $\frac{1}{2}$ of their original size; the amount of ^{15}N incorporated into the proteins of these leaves must, therefore, have been appreciably larger than that incorporated into the old leaves. This result suggests a faster rate of protein replacement in growing than in fully grown leaves. The strong enzymatic action going on in growing leaves leads presumably to an enhanced disintegration and consecutive rebuilding of protein molecules under incorporation of ^{15}N .

The metabolism of nitrogen in the leaves of the buckwheat plant was investigated by a similar method as described above by VICKERY, PUCHER, SCHOENHEIMER and RITTENBERG (1939). In these experiments, the plants were treated for 47 hours only with a culture solution in which the ordinary ammonium chloride was replaced by heavy nitrogen. Disregarding the growth of the leaves during that interval of 47 hours, they conclude from the fact that the "heavy" nitrogen was diluted only 17 times when it was introduced into the leaf protein, that no less than 6 per cent of the protein nitrogen must have undergone replacement by interaction with the simpler nitrogenous substances of the cells which were ammonia and amide nitrogen of augmented isotopic ratio. The protein N replacement of 6 per cent in the course of 2 days is com-

paratively larger than that (12 per cent) found by us in the course of 12 days. When comparing these figures we must envisage the possibility that the replacement rate in buckwheat may be different from that in sunflowers. Furthermore, the rate of replacement is not necessarily proportional with time. Some types of the protein molecules present in the leaves may be renewed at a much faster rate than others.

In a recent paper of VICKERY, PUCHER, SCHOENHEIMER and RITTENBERG (1940), the ^{15}N content of the nitrogen isolated from 7 different nitrogen compounds was compared. Free NH_3 nitrogen was found to be easiest replaceable, while the volatile bases contained the less replaceable nitrogen.

Summary

By making use of heavy nitrogen (^{15}N) as an indicator it was found that in "old" leaves of the sunflower, which did not develop further during the experiment, 12 per cent of the protein molecules present were renewed within 12 days. In growing leaves the replacement of old protein molecules by new ones was found to take place at an enhanced rate.

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COMMENT ON PAPER 93

PROFESSOR UREY supplied us most generously with appreciable amounts of ammonium chloride containing 2.5 per cent heavy nitrogen enabling us to find out whether the protein molecules of the leaves of the sunflower are renewed or not. In the non-growing leaves we found a 1 per cent renewal per day, in growing ones a more rapid renewal rate. RITTENBERG ET AL. (1939) arrived simultaneously at a similar result. Professor SCHOENHEIMER most kindly put at our disposal his mass spectrograph to determine the ^{15}N content of our samples. The determination was carried out by KESTON.

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94. ZINC UPTAKE BY NEUROSPORA

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AMONG the minor constituents of living tissues zinc is one of great interest (see LEHMAN, 1939; BROH-KAHN and MIRSKY, 1948; WARBURG, 1948; BERTRAND, 1948). It constitutes 0.33% of carbonic anhydrase (KEILIN and MANN, 1940), and it is a constituent of uricase which contains 1.3% of zinc (DAVIDSON, 1938; HOLMBERG, 1939) and possibly of other enzymes. It was found to be essential for growth and citric acid formation of *Aspergillus niger* (BERNHAEUER, 1928), for growth and fumaric acid production of *Rhizopus nigricans* (FOSTER and WAKSMAN, 1939) and for growth and subtilin formation by *Bacillus subtilis* (FEENEY, LIGHTBODY and GARIBALDI, 1947).

The presence of 0.2 mgm of zinc in the food of mice is stated by BERTRAND (1948) to prolong the life of the animals from 2—3 weeks to 2—2.5 months.

WASSILJEV (1935) demonstrated that the effect of zinc was not the same for biochemically different strains of *A. niger*, and he went so far as to suggest a characterization of *A. niger* strains based on their reactivity to zinc. FOSTER and WAKSMAN (1939) interpret the function of zinc as catalysing a more complete destruction of the glucose molecule with a consequent greater efficiency of energy utilization by the fungus.

In connexion with studies on the growth of *Neurospora crassa* we determined the amount of zinc necessary to obtain a maximum growth of that mould.

METHODS

Throughout the experiments mycelium from one spore was used. This was produced by crossing the two wild-type strains, nos. 25a and 1A of BEADLE (1945). The culture solution was that described by RYAN, BEADLE and TATUM (1943); it was autoclaved, and the cultures were treated under sterile conditions. Inocula were taken from cultures 4—5 days old.

Labelled zinc as chloride and varying amounts of non-radioactive zinc as sulphate were added to culture solutions at the start of the experiments. From

the specific activities of the zinc present in the culture solution and in the mould and the amount of zinc added to the culture solution, the zinc content of *Neurospora crassa* was calculated. We are much indebted to the Atomic Energy Commission of the U.S.A and the Chief of the Isotope Branch, Dr. Paul AEBERSOLD, for supplying the zinc containing the radioactive isotope ^{65}Zn .

The activity of the ^{65}Zn taken up by *Neurospora* was less than 1 μcurie , and thus the radiation emitted could hardly produce any noxious action on the mould.

After centrifuging and thorough washing the *Neurospora* was dried at 105° , brought into solution by wet ashing, 5 mgm. zinc as sulphate added as carrier and the zinc precipitated with 8-hydroxyquinoline. The precipitate was filtered through a perforated aluminium dish covered by a layer of filter paper, and the dish placed under a Geiger—Müller counter of the type described by MADSEN (1945). A sample of the active ZnCl_2 solution was treated in the same way. By comparing the activities of this 'standard' precipitate of known zinc content and that of the above-mentioned precipitates, the percentage of the zinc originally present in the culture solution which accumulated in the mould was calculated.

RESULTS

The results of some of our experiments, in which each *Neurospora* was grown in 150 ml. culture solution of varying zinc content for 5 days at 25° , are given in Table 1. This table and Fig. 1 show that the presence of 1 part of zinc in about 30,000 parts of dry mould is sufficient for an almost maximal growth, and the presence of $12.5 \mu\text{gm.}$ zinc in 150 ml. culture solution suffices to give that zinc content. It is advisable to add a somewhat larger amount of zinc to the culture solution, e.g.

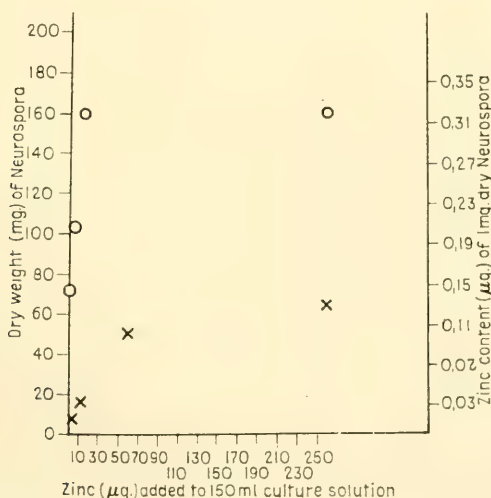


FIG. 1. Effect of varying zinc content of the culture solution on the dry weight (O) and zinc content (X) of *Neurospora* after the lapse of 5 days.

TABLE 1. — AMOUNT OF ZINC TAKEN UP BY *NEUROSPORA*

Zinc in culture solution (μgm)	Wt. of mould (mgm)	Zinc ($\mu\text{gm}/\text{mgm}$ dry mould)	Percentage of the ^{65}Zn content of culture solution present in 1 mgm dry mould
2.5	106.6	0.021	0.84
	106.9	0.013	0.50
	97.8	0.012	0.48
	90.0	0.014	0.55
Average	100.3	0.015	0.59
12.5	175.2	0.033	0.26
	169.7	0.033	0.26
	151.7	0.035	0.28
	144.3	0.040	0.32
Average	158.9	0.035	0.28
50.0	208.6	0.090	0.18
	178.8	0.11	0.21
	168.1	0.10	0.19
	166.7	0.11	0.22
Average	180.5	0.10	0.20
250.0	153.8	0.13	0.033
	172.3	0.12	0.043
	147.7	0.11	0.044
	161.9	0.14	0.055
Average	158.9	0.13	0.049
2000.0	186.3	0.30	0.015
	184.5	0.34	0.017
	158.3	0.38	0.019
	164.0	0.38	0.019
Average	168.3	0.35	0.018

200 $\mu\text{gm}/\text{l.}$ as recommended by RYAN *et al.* (1943) or 450 $\mu\text{gm}/\text{l.}$ as stated by MITCHELL and HOULAKAN (1946).

Figure 1 shows the very pronounced increase in weight of the mould, from 71 to 100 and 159 mgm, after the addition of 2.5 and 12.5 mgm of zinc respectively to the culture solution. In experiments of shorter duration the effect of zinc on the increase in weight was still more pronounced (Fig. 2). After 48 hr 28 mgm were obtained in the absence of added zinc, while adding 30 μgm of zinc to a 150 ml. culture produced 83 mgm of mould.

After 5 hr, a 150 ml. culture solution containing 2.5 μgm zinc produced 100.3 mgm and one with 12.5 μgm zinc, an almost maximal weight of 158.9 mgm. In the former case 1 mgm *Neurospora* contained 0.015 μgm zinc; in the latter roughly twice that amount (0.035 μgm). An increase in the zinc content of the culture solution to 2000 μgm did not increase

the weight of *Neurospora* observed after the lapse of 5 days (Table 1). The capacity of the fungus to take up zinc was, however, not exhausted, as shown by the fact that an eightfold increase in the zinc concentration of the solution resulted in an almost threefold zinc uptake by the mould.

FEENEY *et al.* (1947) determined the amount of zinc to be added to

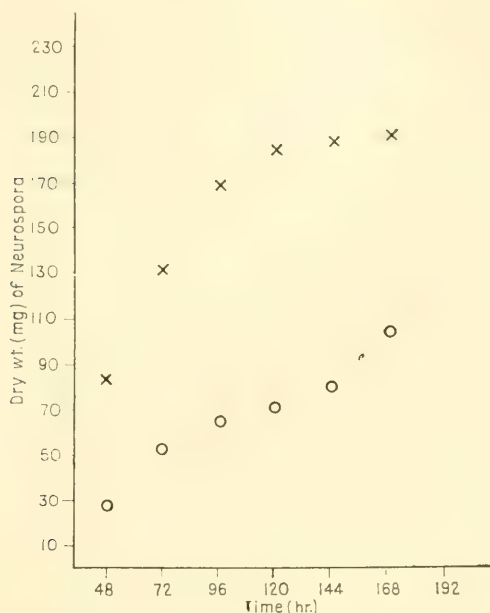


FIG. 2. Dry weight of *Neurospora* without (○) and after (×) addition of 30 μgm. of zinc.

the culture solution in order to obtain maximal growth and maximal antibiotic formation in cultures of *Bacillus subtilis*. This was found to be 0.40 mgm./l., while in the case of *Neurospora crassa* an almost maximal growth was observed by us in the presence of as little as 0.08 mgm./l. of zinc in the culture solution.

Migration of zinc from the *Neurospora* into the culture solution

By making use of isotopic indicators the migration of ions from the plant into the culture solution has been observed in several cases, and the effect on the migration process of unlabelled ions in the solution has also been studied. Minute amounts of labelled lead, when taken up by the roots of *Vicia faba*, could to a large extent be removed by an excess of non-labelled lead added to the nutrient solution, but to a minor extent only if the solution did not contain lead (HEVESY, 1923).

MULLINS and BROOKS (1939) placed cells of *Nitella coronata* first in a solution containing radioactive potassium (^{42}K) and later in solutions of different chlorides. Sodium and lithium were found to be much less effective in removing labelled potassium than potassium itself. We investigated the exodus of labelled zinc into the culture solution both in the absence and presence of zinc in the solution in which the mould containing labelled zinc was placed.

In these experiments *Neurospora* grown for 5 days at 28° , after repeated washing with distilled water, was divided into five approximately equal parts. One part was kept as a control, two parts immersed separately in 150 ml. culture solution containing no zinc for 24 hr at 12° , while two parts were kept under similar conditions in a culture solution containing 30 $\mu\text{gm.}$ zinc.

TABLE 2. — MIGRATION OF LABELLED ZINC FROM *NEUROSPORA* INTO THE CULTURE SOLUTION

S a m p l e	Activity
Control	100
<i>Neurospora</i> after being kept in zinc-free solution for 24 hr at 12°	88, 84
<i>Neurospora</i> after being kept in zinc-containing solution for 24 hr at 12° ...	74, 75
Zinc-free solution after 24 hr.	9, 10
Zinc-containing solution after 24 hr.	14, 19
Penultimate wash water	1

In all our experiments more labelled zinc left the mould when placed in a solution containing zinc. Table 2 shows the results of a typical experiment.

Uptake of cobalt by *Neurospora*

We compared the uptake of Co^{++} (using ^{59}Co as an indicator) with that of zinc by *Neurospora*. Four moulds were grown for 5 days at 28° in the presence of 25 $\mu\text{gm.}$ of cobalt in the culture solution. They were found to take up 0.013, 0.011, 0.011 and 0.011% of the cobalt content of the solution per mgm. of their dry weight (i.e. 0.0033, 0.0028, 0.0028 and 0.0028 $\mu\text{gm.}$). The cobalt uptake corresponds to only about one-twentieth of the uptake of zinc under similar conditions, showing that the moulds do not possess such an effective mechanism for fixing cobalt as they do for zinc.

Summary

1. By making use of labelled zinc (^{65}Zn) the amount of zinc necessary to obtain maximal growth of *Neurospora crassa* was studied.

2. Maximal growth requires that at least 1/30,000 part of the dry weight of the *Neurospora* be composed of zinc, and can be obtained by raising the mould in a culture solution containing 0.08 mgm. or more of zinc/l.

3. When placed in a culture solution containing inactive zinc more labelled zinc migrates from *Neurospora* into the solution at 12° than in the absence of zinc.

4. Cobalt uptake, studied by making use of labelled cobalt (⁵⁹Co), amounts only to one-twentieth of the uptake of zinc under corresponding conditions.

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COMMENT ON PAPER 94

UPTAKE of trace metals of zinc and cobalt, for example, by *Neurospora* can be conveniently studied by making use of labelled metals (paper 94). Maximal growth rate of *Neurospora* was found to require the presence of 0.08 mgm zinc per litre in the culture solution in which the mould is grown.

95. PHOSPHORUS EXCHANGE IN YEAST

G. HEVESY, K. LINDERSTRØM-LANG and N. NIELSEN

From the Institute for Theoretical Physics and the Carlsberg Laboratory of
Copenhagen

THE individual phosphorus atoms present in the leaves of plants have been found⁽¹⁾ for the most part to exchange with great ease within a short time. We extended our experiments to the behaviour of phosphorus atoms present in yeast.

Yeast was grown in a culture solution which, after the lapse of some days, was replaced by a similar solution containing 8.7 mgm of labelled phosphorus per 100 cm³, besides the usual amount of salts and in some cases ten per cent sugar, in others none. The radioactivity of the labelled sodium phosphate was such that 1 mgm P corresponded to 1000 activity units. After the yeast had grown for twenty-four hours in the solution containing labelled phosphorus, it was removed, washed carefully and digested by treatment with sulphuric acid and nitric acid. The phosphorus content of the solution of the yeast was determined both by radioactive measurements and by the usual chemical (colorimetric) analysis.

Yeast grown	Dry weight of yeast (mgm)	Total P found by chemical analysis (mgm)	Total P per mgm dry weight of yeast	Mgm P taken up	
				chem. analysis	radioactive analysis
Initial weight and P content of yeast samples used	108.6	1.375	0.0127		
	108.0	1.384	0.0128		
	108.4	1.361	0.0126		
In labelled P with sugar at 25° .	249.8	3.414	0.0137	2.046	1.966
	260.2	3.407	0.0131	2.034	1.987
	252.3	3.390	0.0134	2.017	2.095
In labelled P with sugar at 0° ..	101.4	1.295	0.0128		0.004
	103.1	1.309	0.0127		0.012
	101.5	1.320	0.0130		0.012
In labelled P without sugar at 20°	89.2	1.369	0.0153		0.044
	88.1	1.345	0.0153		0.054

The results of both determinations for the last set of a long series of experiments are given in the accompanying table. As seen from the later very nearly the same figure for the uptake of phosphorus was obtained by the chemical and by the radioactive analysis. We can conclude from this coincidence that in our experiment no significant exchange of phosphorus atoms took place between the yeast and the culture solution.

Reference

I. G. HEVESY, K. LUNDERSTRØM-LANG and C. OLSEN, *Nature* **137**, 66 (1936); *Ibid.* **139**, 149 (1937).

96. POTASSIUM INTERCHANGE IN YEAST CELLS

G. HEVESY and N. NIELSEN

From the Institute of Theoretical Physics and the Carlsberg Laboratory of Copenhagen

Using radiophosphorus as an indicator, phosphate present in the yeast cells was found to interchange very slowly with the phosphate of the nutrient solution (HEVESY, LINDERSTRØM-LANG and NIELSEN, 1937). Recently, we extended this investigation to the behaviour of potassium, using radiopotassium (^{42}K) as an indicator. In this note, experiments on the uptake and release of radiopotassium by the yeast cells are communicated.

EXPERIMENTAL PROCEDURE

The yeast used in our experiments has been grown for three to five days in beerwort at 25°. A pure yeast culture was applied. The yeast was separated by centrifuging and was purified by repeatedly suspending it in water and centrifuging the suspension. The suspension finally obtained, contained yeast corresponding to a dry substance weight of 1.5 gm per 100 cm³.

The nutrient solution (pH = 4.5) had the following composition: 1.4 gm $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$; 2.0 gm NaH_2PO_4 ; 1.0 gm KCl; 0.8 gm $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$; 1.2 gm $(\text{NH}_4)_2\text{SO}_4$; 0.01 gm $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$; 100 gm saccharose; 0.2 γ biotin; 100 γ aneurin; 2 mgm β -alanin; 2.0 gm citric acid per liter. The KCl was replaced by labelled KCl in those cases in which the intrusion of ^{42}K from the nutrient solution into the yeast cells was investigated.

At the start of the experiment, 1 volume yeast suspension was mixed with 1 volume nutrient solution. The dry substance of the yeast was determined in the usual way by filtering the suspension through a glass filter (1 G 4 Schott) and by drying the yeast at 105°. The potassium content of the yeast and the nutrient solution was determined by the method of WINKEL and MAAS (1936) respectively KOLTHOFF and BENDIX (1939). The samples analysed contained 10–20 mgm potassium. The yeast suspended in nutrient solution was shaken in a thermostat kept at 25°. The samples taken at intervals were sharply centrifuged; they were not washed before analysis in order to prevent loss of potassium by the yeast.

In some of our experiments, the KCl content of the nutrient solution was replaced by labelled potassium chloride; in other experiments, yeast containing labelled potassium was placed in a non-radioactive nutrient solution. The labelled potassium chloride was prepared by bombarding KCl with deuterons accelerated

in the cyclotron. The radiopotassium used in our experiments had an activity amounting to less than 0.001 milliCurie. This activity was found not to influence the growth of the yeast.

Radioactive Analysis

By comparing the radioactivity of the yeast and the nutrient solution samples we obtain the ratio of their ^{42}K content⁽¹⁾. The comparison was carried out by using a Geiger counter. As the weight of the yeast samples was much smaller than the weight of the nutrient solution samples, we mixed some ash non-active nutrient solution to the yeast samples and obtained thus yeast samples having the same weight and almost the same composition as the active nutrient solution samples. The ash sample obtained by this procedure weighed about 120 mgm. It was put in a small aluminium dish of 1.1 cm diameter and placed under the window of the Geiger counter. The procedure described above has the advantage that the absorption of the radioactive (β) radiation in the samples, the activity of which is to be compared, becomes equal and thus can be disregarded.

RESULTS

In some experiments (I, II, and III), labelled yeast was suspended in non-labelled nutrient solution and by analysing the samples taken at intervals the amount of ^{42}K was determined which migrated from the yeast into the nutrient solution. The labelled yeast was obtained by growing yeast in a nutrient solution containing labelled potassium.

In other experiments (IV and V), non-labelled yeast was placed in nutrient solution containing labelled potassium. At different intervals, samples of yeast and nutrient solution were taken and their ^{42}K and their total potassium contents were determined.

TABLE 1. — ANALYTICAL DATA
(a) *Yeast placed in nutrition solution containing ^{42}K*

Time in hours	Fraction	K content of 100 cc in mgm	Distribution coefficient between yeast and nutrition solution		Percentage increase in the dry substance of the yeast
			Total potassium	^{42}K	
0	Nutrition solution ..	21.3	0.43	0	—
	Yeast	9.1			
	Total	30.4			
2	Nutrition solution ..	19.1	0.63	0.38	5.5
	Yeast	12.0			
	Total	31.1			

⁽¹⁾ The "natural" radioactivity of potassium could be neglected in our experiments.

(b) *Yeast containing ^{42}K placed in nutrition solution*

Time in hours	Fraction	K content of 100 cc in mgm	Distribution coefficient between nutrition solution and yeast		Percentage increase in the dry substance of the yeast
			Total potassium	^{42}K	
20	Nutrition solution ..	20.0			
	Yeast	11.7	1.71	0.91	9.9
	Total	31.7			
44	Nutrition solution ..	17.4			
	Yeast	14.1	1.23	1.14	1.0
	Total	31.0			

The analytical data obtained in the first experiment are recorded in Table 1. In this experiment, non-labelled yeast was first placed in a labelled nutrient solution and, after the lapse of 2 hours, a sample of the yeast and a sample of the nutrient solution were analysed. The next step was to place the centrifuged yeast which now contained ^{42}K in a non-labelled nutrient solution. One sample was taken after the lapse of 20, another sample after the lapse of 44 hours. Chemical and radioactive analyses of the samples were then carried out.

The results of our experiments are seen in Table 2. In columns 3, 4 and 5, data on the distribution of ^{42}K between yeast and nutrient solution are given. In those cases, in which non-labelled yeast was placed in labelled nutrient solution, the ratio of the ^{42}K content of yeast and nutrient solution of equal volumina is stated. In the converse cases, the ratio of the ^{42}K content of nutrient solution and yeast is given.

In column 3, the values of the distribution coefficient of ^{42}K are to be seen which we should expect to be present in the case that no exchange of potassium between yeast and nutrient solution took place. Due to the increase of the potassium content of the yeast during fermentation, ^{42}K will *accumulate* in the yeast even in a total absence of potassium exchange between yeast and nutrient solution. If we denote the increment in the potassium content of the yeast by a , and the original ^{42}K content of the nutrient solution by b , the ratio of the ^{42}K content of the yeast and the nutrient solution will work out to be $\frac{a}{b-a}$. In this calculation, the absence of a potassium exchange between yeast and nutrient solution is assumed. In a similar way, the corresponding figures are calculated in those cases in which labelled yeast is placed in non-labelled nutrient solution, in which a decrease will also lead to a change in the ^{42}K content of the yeast without any potassium exchange taking place between yeast and nutrient solution.

In column 4, the distribution coefficient is stated which we should expect in the case of a full interchange taking place between yeast

TABLE 2. — DISTRIBUTION OF ^{42}K BETWEEN YEAST AND NUTRITION SOLUTION

Number of experiment	Time in hours	Distribution coefficient of ^{42}K between yeast and nutrition solution respectively between nutrition solution and yeast ⁽¹⁾			Strength of fermentation	Percentage increase in the dry substance of the yeast
		Assumed absence of potassium exchange ⁽²⁾	Assumed total potassium exchange	Found		
I		Started with active nutrition solution				
	2	0.16	0.63	0.38	slight	5.5
		Started with active yeast				
	20	0.03	1.71	0.91	very strong	3.9
	44	0	1.23	1.14	very strong	1.0
II		Started with active nutrition solution				
	2	0.26	0.90	1.05	very strong	11.3
		Started with active yeast				
	2	0.01	1.04	0.14	fairly strong	— 0.4
III		Started with active nutrition solution				
	2	0.16	0.61	0.14	fairly strong	5.7
		Started with active yeast				
	2	9	1.34	0.14	very strong	1.1
IV		Started with active nutrition solution				
	1.5	0.33	0.88	0.63	very strong	4.3
	23	0.78	1.48	1.61	very strong	56.5
V		Started with active nutrition solution				
	22	0.64	1.19	0.96	very strong	166.8
	22	0.73	1.14	0.85	very strong	174.5

⁽¹⁾ In the first mentioned case we start with radioactive nutrition solution, in the second case with radioactive yeast.

⁽²⁾ Due to increase respectively decrease in the potassium content of the yeast.

potassium and nutrient solution potassium. The figures are calculated from the data of the chemical analyses. In the case of a full interchange, the ratio of the ^{42}K and of the total potassium content of the yeast and the nutrient solution must obviously be the same.

In column 5, the values found for the distribution coefficient are stated.

If no exchange took place the values of column 5 should correspond to the values of column 3 while, in the case of a full interchange, the values of column 5 would correspond to the values of column 4. In the case of a partial interchange, the values of column 3 would lie between the corresponding values of column 3 and column 4. The difference between the values of columns 4 and 5 will decrease with increasing interchange. Since, with increasing interchange, the ^{42}K content of the nutrient solution, respectively of the yeast, is getting more and more "diluted" by non-active potassium moving in the opposite direction we

shall underestimate the extent of the interchange in the case of a strongly pronounced interchange. In this case, the interchange is in fact markedly larger than indicated by the figures of column 5.

In experiments II and IV, one of the values found experimentally is larger than the value expected in the case of a total interchange. This result may be due to experimental errors, an alternative explanation being the following. In the earliest phase of the experiment, potassium of very high specific activity penetrates into the yeast where it gets partly incorporated in a comparatively stable compound which does not get renewed during the experiment.

CONCLUSIONS

As seen in Table 2, already in the course of 2 hours a proportional partition of ^{42}K between nutrient solution potassium and yeast potassium is obtained, indicating that a full interchange has taken place between the potassium present in the solution and the potassium present in the yeast cells in experiment II, in which the yeast has shown a very strong fermentation. In experiment III, in which the fermentation was much weaker, the extent of exchange was a very restricted one. In experiment I, in which only a slight fermentation took place, about half of the yeast potassium interchanged with solution potassium. In experiments taking 22 and 23 hours, respectively, (IV and V), most potassium exchanged.

In all these experiments, the partition of ^{42}K added to the nutrient solution between yeast and solution was followed. In another set of experiments, the opposite process. We have grown yeast in a solution containing ^{42}K and suspended the yeast in a nutrient solution containing inactive potassium. In the course of 2 hours, only, a slight interchange took place (II and III); in the course of 20 hours (I), a very appreciable interchange was observed; after the lapse of 44 hours (I), the interchange was complete. In the two last mentioned cases the fermentation was very strong; in the two first mentioned cases the fermentation was restricted.

A survey of the figures obtained suggests that fermentation promotes interchange between cellular potassium and potassium present in the solution. This result can be interpreted by assuming that potassium permeates the cell wall of the fermenting yeast easier than the cell wall of non-fermenting yeast. An alternative and not less probable explanation is the following: We assume that potassium is partly present in the yeast cells in chemical binding and that the organic compounds containing the potassium degenerate and get resynthesized constantly, and it is in the course of the last mentioned process that ^{42}K has opportunity to be incorporated into the potassium compounds. It is to be expected that, during intense fermentation, the process of renewal of

the potassium compounds, which is presumably intimately connected with the metabolic process going on in the yeast cells, will be strongly enhanced and, correspondingly, the incorporation of ^{42}K in the organic compounds will be accelerated. On this view, it is not so much an increase in the permeability of the cell membrane during intense fermentation which is responsible for the enhanced potassium exchange but the accelerated metabolic rate inside the cell.

It is of interest to remark that PULVER and VERZÁR (1941) quite recently found that there is an intimate connection between carbohydrate metabolism and potassium uptake denoted in this note as "additional uptake" of potassium in contrast to potassium exchange. They found a large amount of potassium to enter the yeast cells after addition of glucose, the potassium leaving again to a large extent after fermentation starts.

Summary

The interchange of potassium between yeast and nutrient solution was investigated by using radiopotassium (^{42}K) as an indicator.

In strongly fermenting yeast, a full interchange was found to take place between the potassium of the yeast and the potassium of the nutrient solution in the course of 2 hours. In most experiments in which the fermentation was slight, a much slower potassium exchange was observed.

The effect of fermentation on the rate of potassium exchange is possibly not so much due to an increase in the permeability of the yeast cell membrane to potassium as to an enhanced rate of resynthesis of potassium containing compounds under fermentation. Such a process will lead to an acceleration of potassium interchange.

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COMMENT ON PAPERS 95, 96

UPTAKE of phosphate into fermenting yeast cells was found under the conditions of the experiment to be an almost one-sided process (paper 95). In strongly fermenting yeast, a full interchange, however, takes place between the potassium of the yeast cells and the potassium of the nutrient solution already in the course of 2 hr. (paper 96). By making use of ^{42}K and also with ^{32}P labelled yeast cells the extrusion rate of potassium and of phosphorus compounds from the yeast cells can conveniently be measured.

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97. NOTE ON THE NUMBER OF POLLEN GRAINS IDENTIFIED IN THE FRUIT OF THE ASPEN

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From the Institute for Research in Organic Chemistry, Stockholm

THE atoms of paternal origin present in the offspring are not confined to the atoms of the fertilizing sperm. The constituents of the numerous spermatozoa ejaculated into the female organism are absorbed and subsequently partly incorporated into the foetus. Do excess pollen grains contribute in a similar way to the formation of the seed, and, should this be the case, to what extent?

In order to clear up this point we produced aspen pollen containing radioactive phosphorus, fertilized female aspen with this and determined the radioactive phosphorus content of the seeds obtained.

EXPERIMENTAL

Three aspen branches were placed shortly before the formation of pollen in glass cylinders each containing about 20 ml of a 0.5 mM sodium phosphate solution of 1 millieurie activity.

The labelled phosphate diffused into the branch and participated in the formation of pollen. Strongly active pollen was obtained after the lapse of 7 days emitting several β -particles per microgram of pollen.

It was of importance to add the labelled phosphate to the solution containing the branch before the formation of pollen, as the uptake of ^{32}P by already existing pollen is very restricted.

That preformed leaves take up appreciable amounts of ^{32}P is seen in Fig. 1, which shows the radioautograph of a leaf on a branch placed in the active solution for 8 days. The leaf, after removal from the branch, was placed on a film covered with a thin layer of cellophane and held in position by a heavy glass plate. After the lapse of 2 days the film was developed. Fig. 2 shows the radioautograph of 3 leaves which were largely grown after the uptake of radioactive phosphate by the branch.

Knowing the activity of 1 microgram pollen and the number of pollen grains weighing 1 microgram, we can calculate the activity of a single grain.

After collecting the fruits about 3 weeks after fertilization, we determined the activity of a known number of grains.

From the ^{32}P content of a seed and that of a pollen grain, we can calculate the number of grains whose ^{32}P content participated in the formation of a seed.

The number of pollen grains in a microgram is determined with a counting chamber as used in the determination of red corpuscles.

A suspension containing about 0.5 mgm pollen per ml was found to be best suited for such determinations.

A few thousand of pollen grains and a few hundred fruits sufficed for the radioactive measurement. Such measurements were carried out partly by com-



FIG. 1. Radioautograph of an aspen leaf. The branch bearing the leaf was placed in a solution containing ^{32}P for 8 days.

paring the activity of a known number of pollen grains and fruits and partly by ashing a known weight of pollen or fruit and precipitating the phosphorus in an aliquot as magnesium ammonium phosphate. The activities of the fractions were then compared.

RESULTS

The first experiment was started on April 3rd, 1944. On April 19th the grains were ready to be investigated. One pollen grain was found to weigh $0.003\ \mu\text{gm}$ and to have an activity of 4.3×10^{-3} counts per minute. *One count per minute thus corresponds to 232 pollen grains.*

A seed was found to weigh 0.04 mgm and to produce 0.036 counts per minute. One count per minute thus corresponds to 28 seeds. From these figures it follows that *one seed contains the labelled phosphorus originally present in 8.3 pollen grains.* A catkin investigated contained 537 seeds and hence as much labelled phosphorus as was originally present in 4,450 pollen grains.

The second experiment carried out in 1945 did not yield a sufficient amount of labelled pollen.

The third experiment was started on February 19th 1946. The aspen branch being kept for 7 days in an active solution. About 0.5 gm pollen was obtained containing about 0.5 per cent of the activity added. The

female branch was fertilized on February 26th—27th, the seeds being collected on March 17th—20th. A poor harvest was obtained, although the number of seeds collected sufficed for the experiment. The comparison of the activities of the seeds and the pollen by Mr. J. OTTESEN led to the following result.



FIG. 2. Radioautograph of leaves from aspen branch placed in a solution containing ^{32}P .

(a) Determination carried out by OTTESEN's micromethod.⁽¹⁾

No. of Dish	Weight of Seed	Number of Seeds
I	4.91 mgm	110.0
II	5.75	128.5
III	4.27	95.5
	14.93	334.0

Weight of pollen..... 1.07 mgm

No. of Dish containing Seeds, Date	Counts/min. Produced by Seeds	Counts/min. Produced by Pollen	^{32}P from μgm Pollen present in 1 Seed
7/4—8/4 II	3.21	1 469	0.0212
8/4—9/4 II	4.11	1 413	0.0242
9/4—10/4 III	2.62	1 351	0.0218
11/4—12/4 I	2.86	1 220	0.0228
12/4—13/4 I	2.76	1 183	0.0226
13/4—14/4 II	2.96	1 129	0.0218
3/4—4/4 II	4.89	1 747	0.0233
4/4—5/4 III	3.67	1 662	0.0247
5/4—6/4 I	4.04	1 599	0.0236
6/4—7/4 II	3.88	1 515	0.0213
7/4—8/4 III	3.34	1 439	0.0260

Average value 0.0230 ± 0.0005

(1) J. OTTESEN, *Acta Physiol. Scand.* **10**, 201 (1945).

(b) Determination carried out by the usual macromethod.

Number of grains investigated = 550

mgm of pollen investigated = 1.61

Date	Seeds (Counts per min.)	Pollen (Counts per min.)	^{32}P from μgm Pollen present in 1 Seed
$8/4 - 9/4$	9.0	1 064	0.0248
$9/4 - 10/4$	8.4	1 017	0.0242
$11/4 - 12/4$	7.93	931	0.0243
$13/4 - 14/4$	7.07	820	0.0252

Average value 0.0246 ± 0.0004

From the above figures it follows that a seed contains an amount of ^{32}P present in $0.0246 \pm 0.0004 \mu\text{gm}$ pollen.

As the weight of one pollen grain was found to be $0.0027 \mu\text{gm}$, one seed had the ^{32}P content of 8.8 pollen grains, this result being almost the same as that obtained in 1944 (8.3 pollen grains).

Summary

Spring aspen branches were placed in a solution containing radioactive phosphate. By this procedure labelled pollen was obtained.

Female aspen branches were fertilized with the labelled pollen and the radioactivity of the seeds obtained was determined.

In the experiment carried out in the spring of 1944, one seed was found to contain the amount of ^{32}P present in 8.3 pollen grains. In experiments made in the spring of 1946 the corresponding figure was found to be 8.8. Thus, in the formation of a seed not only the material of the fertilizing pollen grain participates but also that of a further 7 grains.

COMMENT ON PAPER 97

WHEN fertilizing female aspen branches *in vitro* with ^{32}P labelled pollen, a seed was found to contain as much ^{32}P as was present in from 8.3 to 8.8 pollen grains. This at first sight puzzling result suggests the explanation that a fraction of the fertilizing pollen is metabolized, and some of its phosphorus atoms participate in the building up of seed. The foetus also contains very many more paternal phosphorus atoms than present in the fertilizing sperm.

98. SOME APPLICATIONS OF ISOTOPIC INDICATORS

G. de HEVESY

Nobel Lecture, delivered at Stockholm, December 12, 1944

THE method of isotopic indicators had its ultimate origin in the Institute of Physics at the University of Manchester, which then was under the inspiring leadership of that great physicist, the late Lord (then Professor) ERNEST RUTHERFORD.

The cradle of radium is the Czecho-Slovakian town Joachimstal ; it was from Joachimstal pitchblende ore that Professor and Madame CURIE isolated that element. The Austrian Government, the owners of these mines, generously supplied Professor RUTHERFORD not only with radium, but also with the by-products of radium production, equally important for the worker in the field of radioactivity. One of the most significant by-products is radium D, which has a half-life period of 20 years and is found associated with the very substantial amounts of lead present in pitchblende. The Austrian Government presented to Professor RUTHERFORD several hundred kilograms of such "radiolead". In view of its association with very large amounts of lead, which absorb the radiation emitted by radium D, this precious radioactive material nevertheless proved to be almost useless. When I met Professor RUTHERFORD one day in 1911 in the basement of the laboratory where the radio-lead was stored, he addressed me in his friendly and informal way, saying : "My boy, if you are worth your salt, you try to separate radium D from all that lead." In those days, I was an enthusiastic young man and, on immediately starting to attack the problem suggested to me, I felt quite convinced that I would succeed. However, although I made numerous attempts to separate radium D from lead and worked for almost two years at this task, I failed completely. In order to make the best of this depressing situation, I decided to use radium D as an indicator of lead, thus profiting from the inseparability of radium D from lead. Suppose that we dissolve 1 gm of lead in the form of nitrate in water, add radium D of negligible weight showing a radioactivity of one million relative units (an electroscope being used to measure the activity), and proceed to carry out the most intricate operations with this "labelled" lead. If we then ascertain the presence of one radioactive unit in a

fraction obtained in the course of these operations, we must conclude that 1/1000 mgm of the lead atoms present in the lead nitrate we started from, are now present in the fraction.

Radium D cannot be separated from lead, but it can easily be obtained in the pure form from lead-free radium salt samples or from radium emanation, since radium D is formed in the course of the disintegration of these radioactive bodies and can readily be separated from them. At that time, the Vienna Institute of Radium Research had more radium and radium emanation at its disposal than any other institution. This fact induced me, late in 1912, to start work at the Vienna Institute in collaboration with Dr. PANETH, assistant at that Institute, who himself had made very extensive and abortive trials to separate radium D from lead. The first application of labelled lead⁽¹⁾ was the determination of the solubility of some very slightly soluble lead compounds such as lead chromate and sulphide. Labelled lead chromate was obtained by adding a solution of 100,000 relative units of radium D to lead nitrate containing 10 mgm of lead and converting the nitrate thus labelled into chromate.

After the saturated solution of this compound had been held at the desired temperature in a thermostat for a sufficient time, its composition was ascertained by evaporating a few cubic centimetres to dryness and measuring the activity of the almost invisible residue in the electroscope. From the number of units of radium D found, the amount of lead was calculated, one unit corresponding to 10^{-6} grams of lead; finally, the solubility of the lead chromate in moles per litre ($2 \cdot 10^{-7}$) was computed.

The radioactive method is extremely simple, having the advantage that the presence of foreign ions in no way interferes with the measurements. The method may be applied without difficulty, for example, in determining the solubility of lead sulphate in the presence of calcium sulphate.

Simultaneously with the said experiments, we used labelled lead and labelled bismuth (the radioactive bismuth isotope radium E can easily be obtained from radium, radium emanation, or radio-lead) in an investigation of the manner in which unweighable amounts of metals are precipitated during electrolysis.⁽²⁾ The application of the well-known NERNST formula

$$e = -\frac{RT}{n} \log_n \frac{c}{C}$$

was extended to concentrations of 10^{-8} N and even lower.

On the basis of NERNST's theory, we should expect an interchange to take place between the metal of the electrodes and the ions in solution.

The existence of such an interchange was demonstrated⁽³⁾. While, in the case of lead peroxide, the interchange was found to take place between the outermost layer of molecules of the geometrically calculated electrode surface, in the case of the lead electrodes numerous layers of molecules were found to participate in the interchange process. This finding is to be interpreted as a result of local currents due to variations in the structure of the metallic surface. An interchange between atoms of a lead foil and the lead ions present in a solution was found to occur very rapidly, while the ions adsorbed on colloidal lead particles were found to interchange at a slow rate only.⁽⁴⁾

In contrast to metallic surfaces, PANETH⁽⁵⁾ found that in the case of salt crystal surfaces the interchange was restricted to the uppermost molecular layer of the crystal. On this observation he based an important method for the determination of the surface areas of crystalline powders⁽⁶⁾.

When lead sulphate is shaken with its saturated solutions, a constant kinetic exchange occurs between the molecules of lead sulphate in the solution and those on the surface of the solid. If the solution contains marked molecules, after equilibrium has been attained the numerical ratio of marked molecules on the surface to those in solution is identical to that of total molecules on the surface to total molecules in the solution. As the distribution of the labelled molecules is determined by means of radioactive measurements, and as the lead sulphate content of the saturated solution is evaluated by the usual methods of analytical chemistry, the amount of lead sulphate present in the uppermost molecular layer can be computed. When the weight is known of a uni-molecular layer of lead sulphate of 1 cm² area, the surface of the crystal powder can be calculated from the above data.

Among the numerous applications of radioactive indicators by PANETH I wish to emphasize the importance of his discovery of the existence of bismuth hydride⁽⁷⁾ and lead hydride⁽⁸⁾. After he gained experience regarding the best method of preparation and the stability of radioactive bismuth hydride and lead hydride, he succeeded in preparing these compounds from inactive bismuth and inactive lead, respectively.

SELF-DIFFUSION

The conception of the diffusion of a substance into itself, self-diffusion, was introduced by MAXWELL. No further use was made of this concept until fifty years later, when the method of radioactive (isotopic) indicators was introduced. The possibility of measuring self-diffusion by following the rate of penetration of the lead isotopes ThB or RaD into lead soon suggested the measurement of the self-diffusion in liquid and

solid lead, using ThB or RaD as indicators. The measurements of the self-diffusion coefficient in liquid lead⁽⁹⁾ gave the result anticipated from the known diffusion rates of lead in mercury and other related elements. The diffusion rate in liquids is primarily determined by the radius of the diffusing particle and the viscosity of the liquid: thus, the replacement of a diffusing metal present in small concentration by another related metal will not appreciably influence the rate of diffusion. A very different behaviour was revealed, however, when the self-diffusion in solid lead⁽¹⁰⁾ was measured, using RaD as indicator. In the first experiments carried out in collaboration with GROM, we soldered a piece of radio-lead to the bottom of a rod of ordinary lead, whereafter the system was kept at 280° for 140 days. After the lapse of that time, we cut the system into four equal parts, rolled the four lead pieces into thin plates, and placed them in an electroscope. No diffusion of the radio-lead into the ordinary lead could be ascertained, showing that the self-diffusion rate in lead must be at least several hundred times smaller than that of gold in lead, as determined by ROBERTS-AUSTIN.

This result necessitated the introduction of special methods of great sensitivity for measuring diffusion. Since the rate of diffusion is inversely proportional to the square of the thickness of the layer, we worked out methods for the measurement of the penetration of radioactive lead into ordinary lead layers to a depth of only a few microns. The α -particles emitted by ThB (more correctly, by its disintegration products, ThC and ThC', which, however, attain radioactive equilibrium with the former within a few hours) produce scintillations on a zinc sulphide screen, the number of which is ascertained. The infinitesimal layer of ThB, which is in intimate contact with a lead foil placed below it, is then heated for a few hours to, say, 200°. If a diffusion of the ThB atoms into the lead foil takes place, the count of the scintillations will give a smaller value after the experiment than before. The range of the α -particles in lead being only about 30 μ , a shift of a small percentage of the ThB atoms to depths less than 30 μ will suffice to reduce the counts of scintillations to a noticeable extent. This method, worked out in collaboration with Mrs. OBRUTSHEVA⁽¹¹⁾, was later replaced by a more sensitive and exact procedure applied in diffusion measurements in solid metals, and also salts, in collaboration with SEITH⁽¹²⁾. ThB was condensed on a foil or a single crystal of the metal, and the ionisation produced by radiation emitted by the radioactive body was measured. A slight diffusion of the ThB into the lead after heating sufficed to diminish the ionizing effect registered by an electrometer. Instead of the ionization produced by the α -rays, the ionization produced by recoil particles accompanying the emission of α -rays was measured in some cases. The range of the recoil particles in lead being only about 10^{-5} cm.,

these measurements made possible the determination of diffusion coefficients as small as 10^{-12} cm² per day.

Our measurements led to the result that, while the diffusion coefficient of gold in lead is found to be $5 \cdot 10^{-3}$ cm² day⁻¹ at 165°, the coefficient of self-diffusion in lead at the same temperature is only 10^{-6} cm² day⁻¹, the difference rapidly increasing with decreasing temperature. The change of the value of the coefficient of self-diffusion, D , in lead foils and single crystals is represented by the equation

$$D = 5.76 \cdot 10^5 e^{-27900/RT} \text{ cm}^2 \text{ day}^{-1}.$$

Making use of this formula, we can show that, at room temperature, the atoms will change their places in a piece of lead on the average only once in 10 days.

From the change of the coefficient of self-diffusion with temperature, the heat of activation of the diffusion process, the heat of loosening of the lead lattice, can be calculated. The value obtained and, for purposes of comparison, other thermal data are given in Table 1.

TABLE 1. — THERMAL DATA FOR SOLID LEAD

	kcal. per g. atom
Heat of melting	1.1
Energy content at the melting point	3.5
Heat of lattice-loosening	27.9
Heat of evaporation	36.2

ROBERTS-AUSTIN measured the diffusion rate of gold in solid lead. His measurements gave the first quantitative determination of diffusion rates in solids. The high values he obtained, shown in Fig. 1 (13), led his contemporaries to consider diffusion in solid metals a comparatively rapid process. The introduction of the conception of self-diffusion and the subsequent development led to a very different view and also to the elucidation of the remarkable nature of the gold-lead system investigated by that pioneer metallurgist.

The methods outlined above were also applied to determine the self-diffusion rate of Pb^{++} in solid lead chloride and lead iodide.⁽¹²⁾ The variation of the self-diffusion rates with temperature can be expressed by the equations

$$D = 1.06 \cdot 10^7 e^{-38120/RT} \text{ and } D = 3.43 \cdot 10^4 e^{-30000/RT},$$

respectively. As first shown by NERNST, the ionic mobilities in an electrolyte solution, and hence the conductivity of the solution, can be calculated if the diffusion rates of the ions are known. We can apply the same ideas to solid electrolytes⁽¹⁴⁾ and calculate, for example, the

diffusion rate of Pb^{++} from the conductivity of lead chloride, on the assumption that the electrolytic conductivity of the salt is due solely to the transference of charges by the Pb^{++} . The diffusion rate of Pb^{++} thus calculated is, however, many thousand times larger than the value found experimentally, showing that the chloride ions are almost ex-

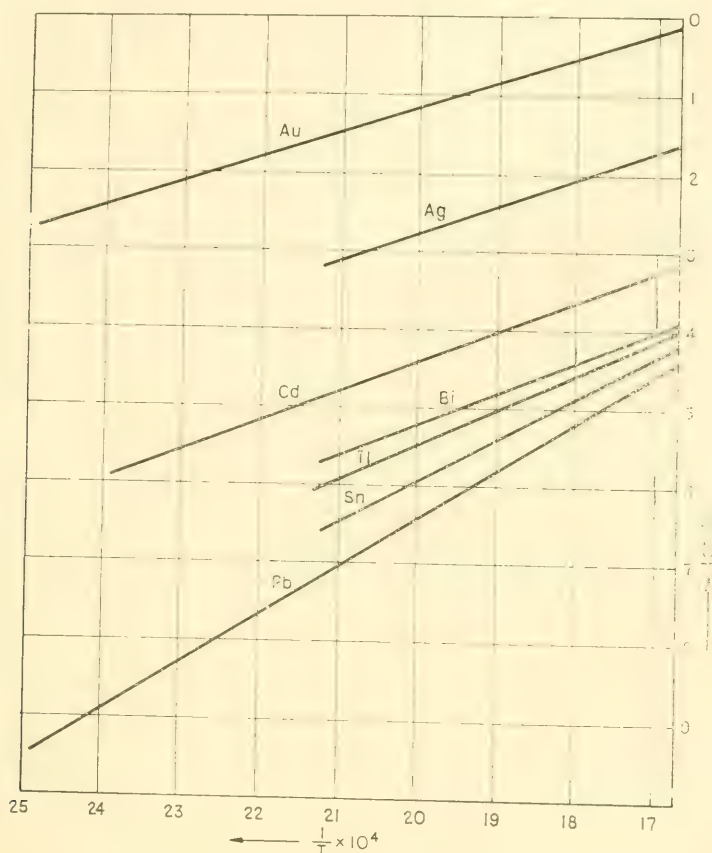


FIG. 1. Diffusion rates of metals in solid lead.

clusively responsible for the conduction of electricity in solid lead chloride. The above data permit the calculation of the transport number of Pb^{++} in solid lead chloride. Not far from the melting point, at 484° , the transport number of Pb^{++} is found⁽¹⁵⁾ to be 10^{-3} , at 273° 10^{-5} , and at 90° only 10^{-10} . By no other method can such small transport numbers be determined in any electrolyte.

In the case of lead iodide, the diffusion rate of Pb^{++} calculated from conductivity data, under the assumption that the whole conduction is due to Pb^{++} , is in good agreement with the measurement of the self-

diffusion rate of Pb^{++} . This shows that, in contrast to the case of PbCl_2 , the conductivity in PbI_2 at high temperatures is due almost exclusively to the transference of charges by Pb^{++} . With decreasing temperature the rôle of Pb^{++} decreases and the transport number of I^- increases accordingly. At 260° , only 40% of the conductivity is due to Pb^{++} and 60 per cent to the I^- ; at 155° the share of the former is only 0.4 per cent. In the case of lead iodide, TUBANDT's beautiful method for the determination of transport numbers could also be applied. The values obtained agreed well with those found by the measurement of self-diffusion.

Formerly, the self-diffusion rates of only lead, bismuth and a few other elements could be determined. These elements have natural radioactive isotopes. The discovery of artificial radioactivity greatly enlarged the possibilities for the determination of self-diffusion rates. By making use of the radioactive bromine isotope, we can determine the self-diffusion rate of Br^- in AgBr just as we determined that of Pb^{++} in PbCl_2 . Working at the Institute for Theoretical Physics at the University of Copenhagen, H. A. C. MCKAY determined the self-diffusion in gold. By the action of neutrons on gold a radioactive gold isotope, having the atomic number 198, can be produced. Neutrons having an energy of about 4 V are strongly absorbed in gold. A thin gold sheet exposed to the action of such neutrons will be more strongly activated on the side first struck by the neutron beam than on the opposite side. When the activated film is heated, the difference in the concentrations of the active gold atoms will decrease and, from the decrease of the activity difference shown by the two faces of the foil, the rate of self-diffusion in gold can be calculated⁽¹⁶⁾.

SVANTE ARRHENIUS' THEORY OF ELECTROLYTIC DISSOCIATION

If we dissolve sodium chloride and the equivalent amount of sodium bromide in water and then separate the two salts by crystallisation, it would have been expected in the time prior to ARRHENIUS that the ions would retain their original partners, the same applying to the bromide ions. According to ARRHENIUS, however, each chloride ion has the same chance of associating with a sodium atom originally bound to chloride as with one initially associated with bromide. The correctness of the much debated views of ARRHENIUS was shown in different ways; the most direct proof, however, was provided through the application of isotopic indicators⁽¹⁷⁾. When equivalent amounts of PbCl_2 and labelled $\text{Pb}(\text{NO}_3)_2$ (or vice versa) were dissolved and the two compounds were separated by crystallization, the labelled lead ions were found to be equally distributed between chloride and nitrate ions.

Very different results were obtained in all cases in which the lead atoms were joined to carbon. Between lead chloride and lead tetraphenyl in pyridine, between lead nitrate and lead tetraphenyl in amyl alcohol, and between lead nitrate in aqueous ethyl alcohol, no change in the places of lead atoms could be detected, although in every combination investigated one of the molecular types was capable of electrolytic dissociation.

The lack of interchange of atoms present in organic binding [hydrogen atoms bound to oxygen or nitrogen being an exception, as shown by BONHOEFFER⁽⁸¹⁾], such as that of carbon atoms in glycogen or phosphorus atoms in lecithin with other carbon and phosphorus atoms respectively, was found to be of great significance for the application of isotopic indicators in biochemical research. Owing to the absence of such an interchange, the presence of labelled carbon atoms in glycogen molecules, or of labelled phosphorus atoms in lecithin molecules, extracted from the organs, proved that a synthesis of these molecules took place after the labelled atoms were administered. This principle enables us to distinguish between "old" and "new" molecules and to determine the rates at which molecules of the different compounds are built up and carried to the different organs.

A prompt interchange of the electrical charges between Pb^{++} and Pb^{+++} ions was found to take place in experiments where plumbeous acetate and labelled plumbic acetate (or vice versa) were dissolved in glacial acetic acid and then separated by crystallization⁽¹⁸⁾. The same holds for Tl^+ and Tl^{+++} ions⁽¹⁹⁾. An interchange of lead atoms takes place between fused lead and fused lead chloride, lead oxide or lead sulphide⁽²⁰⁾.

After artificially radioactive isotopes became available as indicators, interchange processes were studied in numerous cases. A rapid interchange of charges was found to take place between Fe^{++} and Fe^{+++} , Cu^+ and Cu^{++} , etc.⁽²¹⁾.

ANALYTICAL CHEMISTRY

Analytical chemistry proved to be a fruitful field for the application of isotopic indicators. A knowledge of the total lead content of the earth's crust, for example, is of considerable chemical interest. In view of the small lead content of the average rock sample, the quantitative determination of its lead content involves some difficulties. These have been eliminated by making use of an isotopic indicator⁽²²⁾. An amount of radium D, known in relative radioactive units, is added to the solution of the rock sample; the radium D is then recovered by electrolysis as peroxyde. If 100 per cent of the added radium D is recovered, we may

expect 100 per cent of the lead present in the sample to have been recovered as well. If only 50 per cent is recovered, for example, we have to multiply the amount of lead recovered by 2 in order to arrive at a correct analytical figure. The indicator method thus allows a correction for the shortcomings of the analysis. Such corrected analytical figures are seen in Table 2.

TABLE 2. — LEAD CONTENT OF IGNEOUS ROCKS

Rock types	gm lead per gm rock precipitated by electrolysis	Percentage Ra D recovered by electrolysis	gm lead per gm rock. Corrected value
Gabbros and related types (composite of 67 samples)	$4 \cdot 10^{-6}$	80	$5 \cdot 10^{-6}$
Essexites and related types (composite of 40 samples)	$7 \cdot 10^{-6}$	80	$10 \cdot 10^{-6}$
Soda-granites and soda-syenites (composite of 26 samples)	$9 \cdot 10^{-6}$	73	$11 \cdot 10^{-6}$
Granite rocks (composite of 58 samples)	$18 \cdot 10^{-6}$	53	$30 \cdot 10^{-6}$
Basalt, Giant Causeway	$4 \cdot 10^{-6}$	100	$4 \cdot 10^{-6}$

Instead of adding radium D to the solution to be analysed, we may add lead labelled by the presence of some radium D, for example 100 mgm of lead having an activity of 1000 units. If we subsequently isolate 10 mgm of lead from the solution, this lead should show an activity of 100 units, under the assumption that the original sample does not contain lead. If the activity of the isolated 10 mgm of lead is, for example, found to be 83 only, we have to conclude that the sample contains lead amounting to 20 mgm.

In recent years, isotopic indicators have found an extended application in biochemical analyses. SCHOENHEIMER and his colleagues⁽⁸²⁾ determined the leucine content in the protein of the rat by adding to the hydrolysate a known amount of leucine containing heavy nitrogen. This tracer was also used⁽⁸³⁾ in the investigation of the occurrence of the amino acids of the *d* series in cancer proteins; while CHARGAFF, ZIFF and RITTENBERG⁽²⁵⁾ used bases containing a known amount of ^{15}N in the analysis of the nitrogenous constituents of tissue phosphatides. Amino acids containing deuterium as indicator were used by USSING⁽²³⁾ and the same tracer was applied by RITTENBERG and FOSTER⁽²⁴⁾ in their determination of the palmitic acid content of rats' fat.

EARLY BIOLOGICAL APPLICATION

In contradistinction to the animal body, the uptake of mineral constituents by the plant is not followed by a loss of such constituents, and it was formerly considered that the ions taken up by the roots of the plant did not migrate in the opposite direction at all. The application of isotopic indicators, however, has shown that this is not the case. Ions taken up by the plant can be removed by an exchange process under the action of other ions present in the soil or in the nutrient solution. It was already found in 1923 that minute amounts of lead, labelled by the admixture of the lead isotope thorium B, when taken up by the roots of *Vicia faba*, could to a large extent be removed by an excess of non-labelled lead added to the nutrient solution⁽²⁶⁾. Most other ions were found to be much less effective in removing the labelled lead ions from the plant.

In recent years, the behaviour of essential constituents of plants has been investigated, making use of artificial, radioactive ions as indicators; similar results were obtained. MULLINS and BROOKS⁽²⁷⁾ placed cells of *Nitella coronata* first in a solution containing radioactive potassium and later in solutions of different chlorides. Sodium and lithium were found to be much less effective in removing labelled potassium than potassium itself, whereas rubidium was more effective. JENNY and OVERSTREET⁽²⁸⁾ and BROYER and OVERSTREET⁽²⁹⁾ found that ionic exchange could take place during periods of, and under conditions favourable for, active solute uptake. It was also observed⁽³⁰⁾ that for each six phosphate ions taken up by the roots of growing wheat seedlings, one phosphate ion migrated from the roots into the nutritive solution.

Early in the twentieth century, the application of bismuth compounds in syphilis therapy came to the fore. This induced CHRISTIANSEN, LOMHOLT and HEVESY⁽³¹⁾ to investigate the absorption, circulation and excretion of labelled bismuth preparations. LOMHOLT⁽³²⁾ succeeded in showing that, of all the preparations investigated, bismuth hydroxide suspended in oil was most suitable for therapeutic application.

Successes achieved by BLAIR-BELL in cancer therapy, using lead compounds, induced the investigation of the partition of labelled lead compounds between normal and tumorous tissue.⁽³³⁾ Though this work gave a negative result, it nevertheless proved to be of great importance in the future development of isotopic indicators. It was in the course of these investigations that SCHOENHEIMER became familiar with the method of isotopic indicators, which he applied several years later with such great success in the study of fat and protein metabolism and of numerous related problems. Never were more beautiful investigations carried out with isotopic indicators than those of the late Professor

SCHOENHEIMER, whose untimely and tragic death is much to be deplored. The discussion of the numerous important results obtained by SCHOENHEIMER and RITTENBERG and their collaborators⁽³⁴⁾ lies, however, beyond the scope of this lecture.

HEAVY WATER

In 1931, UREY discovered deuterium, an isotope of hydrogen.⁽³⁵⁾ This important discovery made possible the labelling of hydrogen. Deuterium is not an ideal indicator, its properties differing appreciably

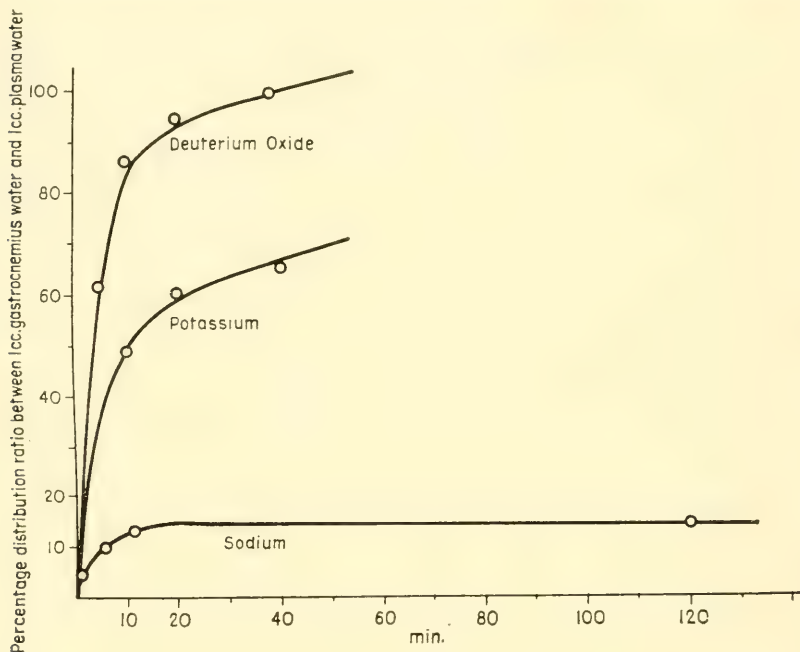


FIG. 2. — Percentage distribution ratio of labelled sodium, potassium and deuterium oxide between plasma water and muscle water of equal weight.

from those of hydrogen. The latter has a unique position: it is the sole element met with, though only transitorily, as a naked nucleus in chemical reactions. Chemical forces do not suffice to remove all electrons from any other element. Differences in the structure of the nucleus will therefore make themselves more noticeable in the chemical behaviour of hydrogen isotopes than in the case of any other element. Furthermore, the difference between the mass of the hydrogen atom and that of the deuterium atom amounts to as much as 100 per cent, while, for example,

the corresponding difference between a ^{31}P and a ^{32}P atom is only 3 per cent. The very small difference in the chemical properties of ^{31}P and ^{32}P remains at the present time within the errors of our experiments, whereas between hydrogen and deuterium the difference is quite appreciable. The same applies to H_2O and D_2O . Dilute "heavy" water, however, contains mostly DOH molecules which exhibit in their chemical behaviour a very great resemblance to HOH .

In the study of the circulation of water in the organism, dilute heavy water can therefore safely be used as an indicator. In the determination of the life period of water molecules in the human organism, water containing $\frac{1}{2}$ per cent heavy water was used⁽³⁶⁾. While a small percentage (0.1) of the water was found to be excreted in so short a time as 26 minutes, the average life of the water molecules in the organism was found to be 13.5 days. In the excreted water, molecules were thus found which were taken in both a few minutes and several months before the investigation. By extrapolation, however, we arrive at the result that, though the number of water molecules present in an adult organism amounts to as much as c. 10^{27} , the adult organism no longer contains a single water molecule taken up at birth.

The rate of admixture of administered water with the water present in the body was investigated in experiments on rabbits⁽³⁷⁾ and guinea pigs⁽³⁸⁾. While the water reaching the circulation was found to enter into exchange equilibrium with the extracellular water (about $\frac{1}{4}$ of the weight of rabbit) in the course of a few minutes, the penetration of the water molecules into the cells took some time. As is seen in Fig. 2, about 30 min passes before the exchange equilibrium is reached between the water administered and extracellular and intracellular water present in the tissues of the rabbit. In the guinea pig, 73 per cent of the water in the blood is exchanged for extracellular water every minute.

Time does not permit me to discuss the extended application of heavy hydrogen, heavy carbon and heavy nitrogen, and to treat the numerous important results obtained by the use of these isotopes as indicators.

APPLICATION OF ARTIFICIALLY RADIOACTIVE ISOTOPES

During the lengthy operations preceding the early experiments of self-diffusion in lead, we often discussed the great progress which might be expected if radioactive indicators of the common elements were made available to chemical and biological research. This wish, which seemed utopian in those remote days, was fulfilled by FRÉDÉRIC JOLIOT and IRÈNE JOLIOT-CURIE's⁽³⁹⁾ fundamental discovery of artificial radioactivity, followed by FERMI's⁽³⁹⁾ work leading to the discovery of many more artificially radioactive isotopes. Soon after the announce-

ment of these discoveries, we prepared the radioactive phosphorus isotope ^{32}P by neutron bombardment of carbon disulphide and used this isotope in collaboration with CHIEWITZ⁽⁴⁰⁾ in the study of phosphorus metabolism. In these experiments, 10 litres of carbon disulphide were used to absorb most of the neutrons emitted by a mixture of radium and beryllium kindly put at our disposal by Professor NIELS BOHR. This precious neutron source being a gift by his friends to his fiftieth birthday. The ^{32}P formed was extracted by treatment with diluted nitric acid or with water, the carbon disulphide being immediately available after the extraction for further neutron irradiation.

A few other radioactive isotopes, such as the radio-halogens can also be prepared by similar simple and convenient procedures. This is, however, not the case with the majority of radioactive isotopes. These were prepared in amounts sufficient to be utilized in indicator-work only after the discovery of the cyclotron⁽⁴¹⁾. LAWRENCE's highly significant discovery also made available radiophosphorus preparations of very much greater activity than could be obtained from neutron-sources containing as much as several grams of radium. The number of neutrons produced by the Berkeley cyclotron was stated by BIRGE⁽⁴¹⁾ in 1939 to correspond to the number of ions produced by 100 kgm of radium ; since that date, a still more powerful cyclotron has been brought into use. In our later investigations, radiophosphorus generously put at our disposal by Professor NIELS BOHR, LAWRENCE and SIEGBAHM was used.

The preparations of radioactive isotopes of numerous elements prepared in the Radiation Laboratory at Berkeley and in other laboratories found an extended application as indicators. Radioactive iron prepared at Berkeley, for example, was used by HAHN, WHIPPLE and their colleagues⁽⁴²⁾ in extended studies of iron metabolism.

The application of cyclotron-prepared radio-carbon⁽⁴³⁾ revolutionized our views of the fundamental process of photosynthesis.

Radio-iodine⁽⁴⁴⁾ found an extended application in the study of the formation of thyroxine and diiodotyrosine ; it led, *inter alia*, to the important finding that some thyroxine is formed in the organism even after total extirpation of the thyroideae.⁽⁴⁵⁾

Radio-phosphorus found, however,⁽⁴⁵⁾ the most extensive application. This was due not only to the convenient mode of production and period of decay of this material, together with the convenient absorability of the rays emitted by it, but mainly to the important part which phosphorus plays in a very great number of metabolic processes. These include skeleton formation, metabolism of carbohydrates and fats, cell division, and many other processes. The discussion of the rôle of phosphorus in metabolic processes is therefore well-suited to demonstrate different applications of isotopic indicators in biological research. We

shall therefore now describe some applications of radiophosphorus. These examples represent only a small percentage of the investigations in which radio-phosphorus has been used as an indicator ; many of the results to be discussed were obtained in Copenhagen.

RADIO-PHOSPHORUS

Owing to the great sensitivity of the Geiger-Müller counter, which registers ^{32}P with an activity of only 10^{-6} microcurie, some of the radiophosphorus administered can soon be located in all organs. Table 3 shows the distribution of ^{32}P in the organs of the rat 4 hours after subcutaneous injection of labelled sodium phosphate.⁽⁴⁷⁾

TABLE 3. — DISTRIBUTION OF ^{32}P BETWEEN DIFFERENT ORGANS IN RAT, 4 HOURS AFTER SUBCUTANEOUS INJECTION OF LABELLED PHOSPHATE

Weight of the rat: 188 gm

O r g a n	Per cent ^{32}P present	Specific activity
Bones	22.6	0.020
Muscles	18.7	0.191
Liver	17.6	0.475
Digestive tract	15.9	0.365
Skin	11.1	0.192
Lungs and heart	6.3	0.317
Blood	2.5	0.558
Kidneys	2.4	0.370
Spleen	1.3	0.256
Brain	0.02	0.032

While 4 hours after the administration most ^{32}P is found in the skeleton, muscles, liver and the digestive tract, with increasing time more and more ^{32}P becomes incorporated with the skeleton. 98 days after the start of the experiment, 92 per cent of all ^{32}P present in the rat, which corresponds to about one half of the total amount administered, is found in the skeleton. This result may be seen in Table 4. Most phosphorus taken up with the food, in so far as it is not excreted, ultimately finds its way into the skeleton, where it replaces "old" phosphorus which interchanges with the phosphorus present in other organs or is excreted.

From these results, however, no conclusions can be drawn concerning the extent of renewal of the skeleton, as the labelled phosphorus, i.e. phosphorus administered throughout the experiment, may be incorpo-

rated wholly or principally in the upper molecular layers of the apatite-like crystals which form the mineral constituents of the skeleton. We can determine the extent of renewal of the bone mineral phosphorus by comparing the ^{32}P content, i. e. the radioactivity of mgm bone mineral P, with the radioactivity of 1 mgm free plasma P. Were the bone phosphorus entirely renewed in the course of the experiment, the ^{32}P would be distributed equally between the free P atoms of the apatite-like bone crystallites and the free P atoms of the plasma, the latter being the direct or indirect source of the bone phosphorus. If only 1 per cent of the bone apatite P were renewed in the course of the experiment,

TABLE 4. — PERCENTAGE TOTAL ^{32}P FOUND IN SOME ORGANS OF RATS

Organ	Time after distribution of ³² P						
	hours		days				
	1/2	4	10	20	30	50	98
Muscles	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Total skeleton	19.1	23.4	43.1	43.1	51.8	76.5	92.0

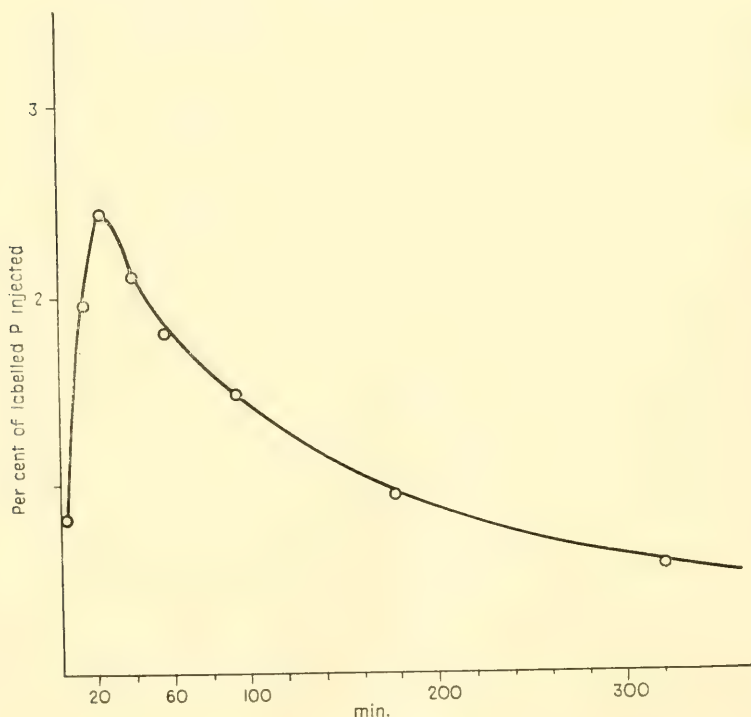


FIG. 3. Change in the specific activity of the plasma inorganic P after subcutaneous injection of labelled phosphate into a rabbit

the specific activity of the bone apatite P would be only 1/100 of that of the free plasma P.

The determination of the degree of renewal based on the said administration is made difficult by the fact that the specific activity of the free plasma phosphorus varies throughout the experiment. After ad-

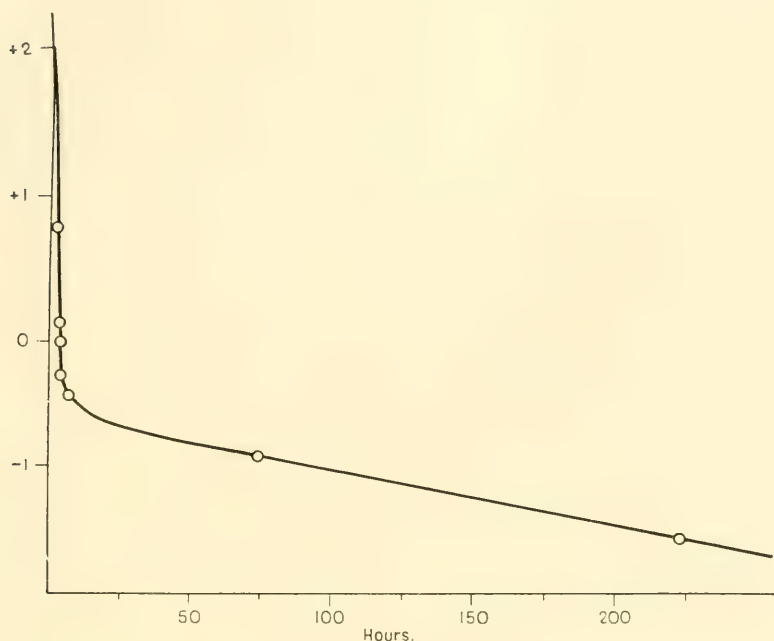


FIG. 4. — Change with time in the logarithm of the labelled P content of the plasma after intravenous injection of labelled phosphate into a rabbit.

ministration by the mouth or by subcutaneous injection, the specific activity first increases and subsequently decreases with time, while after administration by intravenous injection it first decreases very rapidly, and later at a moderate rate, as seen in Figs. 3 and 4.⁽⁴⁸⁾ The determination of the extent of renewal of the mineral P in the skeleton is much facilitated by keeping the free plasma P activity at a constant or almost constant level. This can be attained by repeated injections of varying amounts of labelled phosphate throughout the experiment. Making use of this technique, the data given in Table 5 were obtained for the extent of renewal of the different parts of the skeleton of the adult rabbit in the course of 50 days⁽⁴⁹⁾. As seen in Table 5, 70 per cent of the epiphysis and 93 per cent of the diaphysis remained unchan-

ged after this period, while 29 and 7 per cent respectively were renewed not once, but, at least to some extent, repeatedly.

The restricted extent of renewal of the skeleton is due to the fact that while the P atoms of the uppermost molecular layer of the bone apatite crystals can promptly interchange with the free P atoms of the plasma (actually not the P atoms, but the phosphate ions interchange),

TABLE 5. — EXTENT OF RENEWAL OF THE MINERAL CONSTITUENTS OF THE BONE
IN THE COURSE OF 50 DAYS

	Percentage renewal
Femur epiphysis mineral P	29.7
Femur diaphysis mineral P	6.7
Tibia epiphysis mineral P	28.6
Tibia diaphysis mineral P	7.6
Costa mineral P	27.5
Femur phosphatide P	100

a renewal of the main part of the apatite P can take place only when the crystal is dissolved and when crystals are formed from the plasma ; from a labelled plasma, labelled crystals are formed. This "biological"

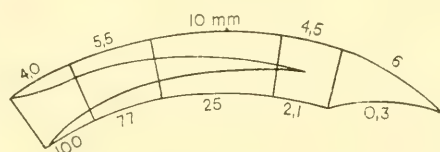


FIG. 5. — Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus. The figures below give the relative amounts of labelled phosphorus present in 1 mgm of fresh tissue in the section in question. The figures above give the length of the section in mm

recrystallization of the skeleton crystallites is a comparatively slow process. Moreover, we have to consider that if only the outer part of the crystal is renewed, this process can often be repeated without affecting deeper molecular layers of the crystal.

No data are available concerning the extent of renewal of the human skeleton ; the relative rates of renewal of different parts of the skeleton were, however, determined by ERF⁽⁵⁰⁾.

The problem as to whether and to what extent the P atoms of the dental enamel are renewed has been a subject of extensive investigations⁽⁵¹⁾ which led to the result that, though some ^{32}P is found to be present in the enamel after administration of labelled phosphate, the extent of renewal of the enamel phosphate is almost negligible. Regarding the extent of replacement of the phosphorus present in the constituents of the dentine, about one millionth part of the food phosphorus was found to be located in the mineral constituents of the dentine of each tooth.

The bone tissue growing in a labelled organism is bound to become labelled. Of the labelled phosphate administered by mouth, after the lapse of 3 days 2 per cent was found to be present in the rapidly growing incisors of the rat⁽⁵²⁾. As seen in Fig. 5, these phosphate ions are mostly found in the incisal part of the incisor, though a minor part are located at the apical end remote from the pulpa.

PERMEABILITY INVESTIGATIONS

The above-mentioned rapid decrease in the plasma activity following intravenous administration of ^{32}P is to a large extent due to the interchange of plasma phosphate with the phosphate of the extracellular fluid. From this fact it follows that the capillary wall is readily permeable to phosphate; similar results were obtained for the other labelled ions investigated. Sodium ions, which are mainly confined to the extracellular space, enter into exchange equilibrium with the plasma sodium within 20 minutes^(53, 84, 85). This may clearly be seen from Fig. 6.⁽⁵³⁾ For potassium and for phosphorus, elements mainly located in the tissue cells, a longer time is required for the attainment of such equilibrium⁽⁵⁴⁾. The low rate at which exchange equilibrium between the cellular and extracellular phosphorus is reached in the animal organism is mainly due to a low rate of renewal of large parts of the skeleton.

As seen from the above examples, the method of isotopic indicators can be utilized with advantage in permeability investigations. It is with the aid of isotopic indicators that we best can measure the permeability of phase boundaries, since other methods do not indicate solely the resistance of the phase boundary to the penetration of ions, but a more complex phenomenon. Prior to the application of isotopic indicators, the high potassium content of the erythrocytes of most species and their low sodium content were interpreted as being due to the impermeability of the erythrocyte membrane to potassium and sodium ions. The application of isotopic indicators, however, has disclosed the fact that potassium ions in the erythrocyte interchange quite easily⁽⁵⁵⁾

with those present in the plasma, and the same applies to the sodium ions. The high concentration of potassium and low concentration of sodium found in the erythrocytes of most animals can thus not be explained as being due to an impermeability of the corpuscle membrane to these ions.

Not only the resistance of phase boundaries to labelled ions, but also that to molecules of different kinds, can be measured with the aid of isotopic indicators. The rates of interchange of phosphatides present

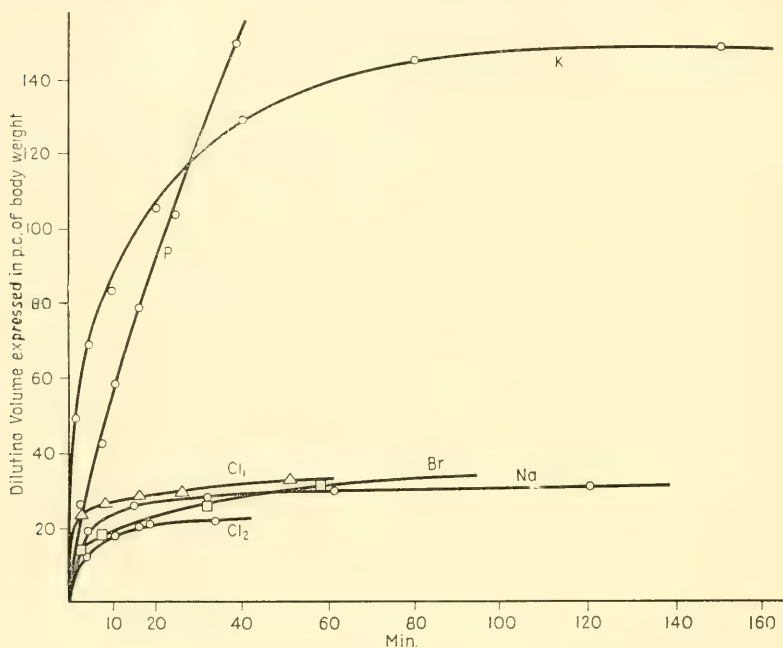


FIG. 6. — Rate of disappearance of various labelled ions from the plasma.

in the plasma and in different organs were determined in the following way⁽⁵⁶⁾. Labelled phosphate was administered to a rabbit. After the lapse of 2 days, when the plasma contained an appreciable amount of labelled phosphatides, part of the plasma of another rabbit (rabbit II) was replaced by the labelled plasma. On following the decrease with time in the activity of the phosphatides extracted from the plasma of rabbit II, it was found that half of the plasma phosphatide molecules had interchanged in the course of few hours with phosphatide molecules present in the organs of the rabbit. An investigation of the activity of the phosphatides isolated from the organs led to the result that a very substantial part of the labelled phosphatide molecules was found in the liver.

RATE OF FORMATION

The rate of formation of labelled organic phosphorus compounds differs much for various compounds and varies greatly with the organ in which they are located. The labile P of adenosine triphosphate, for example, is renewed at a very remarkable rate^(57, 86), the second P atom being renewed somewhat more slowly than the terminal atom⁽⁵⁸⁾. Hexose monophosphate, present in the red corpuscles, was found to be largely renewed within a few minutes.⁽⁵⁷⁾ The formation of labelled phosphatides takes place in the liver and in the intestinal mucosa of the rabbit at a much more rapid rate than in the brain and more quickly than in any other organ⁽⁵⁹⁾. Desoxyribose nucleic acid, on the other hand, shows a behaviour opposite to that of the phosphatides. The extent of its formation in the liver of adult rats is very low^(60, 61, 87), amounting to only about 0.1 per cent in the course of 2 hours. In the spleen and in the intestinal mucosa, the renewal of the desoxyribose nucleic acid is 20 and 30 times, respectively, more rapid than in the liver. High figures for the rate of formation were found in rapidly growing tissue.

X-rays were found to obstruct the formation of labelled desoxyribose nucleic acid molecules⁽⁶¹⁾. During irradiation with X-rays for 2 hours, applying a dose of 15 r/min, the formation of new (labelled) nucleic acid molecules in JENSEN's sarcoma was found to be reduced to about $\frac{1}{2}$ of its normal value. In the study of the reduction in labelled nucleic acid formation under the action of X-rays, a new line of attack was opened in the study of the action of such radiation on cell division.

We calculate the extent of renewal of the compound in question, for example creatine phosphoric acid, by comparing the specific activity of the creatine phosphorus at the end of the experiment with the average specific activity of the free phosphorus present in the tissues cell during the experiment. This calculation is based on the assumption that the labelled free phosphate, or the phosphate of a donor whose P enters rapidly into exchange equilibrium with the free P present in the cells, is incorporated in the creatine phosphate molecule present in the cells of the organ investigated. In the case of phosphatides, the possibility cannot be excluded^(45, 46) that a precursor of the phosphatides molecule is formed at a comparatively slow rate. In such a case, the calculation of the extent of the renewal of the phosphatide molecules would necessitate knowledge of the specific activity of the precursor P. The renewal figures obtained must therefore be interpreted with caution. Another more pertinent reason for the cautious interpretation of the results obtained is that the molecules of some organic compounds may possibly be built up within the phase boundary, where the specific activity of the free P may appreciably differ from that of the intracellular free P.

While we measure the rate of renewal of phosphatide molecules with respect to their phosphate content by employing ^{32}P as an indicator, the rate of renewal of the fatty acid constituents is determined by the use of deuterium⁽⁶²⁾ as indicator, and the new-formation of the choline content by applying ^{15}N as a tracer⁽⁶³⁾. A molecule can clearly be renewed in various ways.

SITE OF FORMATION OF PHOSPHORUS COMPOUNDS IN THE ORGANISM

Origin of Yolk Phosphatides

We shall first consider the site of formation of some constituents of the hen's egg. Where in the organism are the phosphatides found in the yolk synthesized? This question can be answered by comparing the specific activity of phosphatide P extracted from the yolk and from the different organs a few hours after administration of labelled sodium phosphate⁽⁶⁴⁾.

The results of an experiment in which the hen was killed 5 hours after subcutaneous injection of labelled P are seen in Table 6. The specific activities of the yolk phosphatide and the ovary phosphatide P were

TABLE 6. — SPECIFIC ACTIVITY OF PHOSPHATIDES
EXTRACTED FROM THE ORGANS OF A HEN

Organ	Relative specific activity (Activity of inorganic plasma P = 1)
Liver	0.54
Plasma	0.43
Ovary	0.039
Yolk	0.0035
Intestine	0.11
Spleen	0.1

very low, showing that only a small part of the molecules present in the said phosphatides had been formed within the last 5 hours. The plasma phosphatide P had a much higher specific activity than those extracted from the ovary and the yolk, while the liver phosphatide P had a higher specific activity than the plasma phosphatide P. The gradient indicating the presence of phosphatide molecules formed within the last 5 hours, thus falls off in the direction from the liver, through the plasma, into the ovary.

The conclusion that the formation of the phosphatide molecules of the plasma mainly occurs in the liver^(59, 64, 65) is strongly supported by the results obtained in the study of fat phosphorylation in the hepatectomized dog by CHAIKOFF and his colleagues.⁽⁶⁶⁾ These authors injected labelled sodium phosphate intravenally immediately after removal of the liver. Practically no phosphatide ^{32}P was recovered in the plasma as late as 3–6 hours after extirpation of the liver; at these times 0.4 per cent of the injected ^{32}P had been incorporated into phosphatides of both kidneys and about an equal amount into the whole small intestine.

Considerations similar to those applied to the origin of the phosphorus compounds of the yolk were used in an investigation of the phosphorus compounds of milk.⁽⁶⁷⁾ As seen in Table 7, the milk phosphatides were found to have a much higher specific activity than the plasma phosphatides, indicating that the phosphatides must enter the milk from a source other than the plasma and must thus have been synthesized to a large extent in the mammary gland. The determination of the specific activity of the mammary gland phosphatides revealed a very high value, even higher than those found for the kidney and liver phosphatide P.

TABLE 7. — SPECIFIC ACTIVITY OF THE PHOSPHATIDE
P EXTRACTED FROM THE ORGANS OF A GOAT $4\frac{1}{2}$
HOURS AFTER ADMINISTRATION OF LABELLED
SODIUM PHOSPHATE

Fraction	Specific activity
Milk	0.09
Plasma	0.02
Corpuscles	0.01
Mammary gland	0.13
Liver	0.09
Kidney	0.11
Plasma inorganic P	1.48

One often encounters the view that the milk fat originates from the phosphatides of the blood, which are decomposed into fatty acid and inorganic P in the mammary gland. The inorganic P present in the milk should, according to this view, originate from phosphatide P. The fat content of goat's milk amounts to about 3 per cent. Taking the ratio of fatty acid to phosphorus to be 20: 1 in plasma phosphatides, the production of 3 per cent fatty acid from phosphatides would set free 0.15 per cent of inorganic phosphorus. This being about the inorganic P content of the milk, almost all inorganic P of the milk should originate

from plasma phosphatide. A few hours after the administration of labelled phosphate, the milk phosphatides are only slightly active, while the milk inorganic P shows a strong activity. This is a decisive argument against the above view. The high activity already found for the milk inorganic P in the early stages of the experiment, is only compatible with the assumption that the milk inorganic P is derived from the plasma inorganic P. The latter acquires a high activity soon after subcutaneous injection of labelled sodium phosphate.

As a further example we may mention the origin of the phosphorus compounds in the chick embryo⁽⁶⁸⁾. 0.1 cm³ physiological sodium chloride solution containing traces of labelled sodium phosphate was injected into fertilized eggs. Several days after incubation, the phosphatides and other phosphorus compounds were isolated in turn from the embryo and the yolk, their activity and their phosphorus content being determined. As seen in Table 8, the specific activity of the embryo phosphatide P is very different from that of the yolk phosphatide P.

TABLE 8. — SPECIFIC ACTIVITY OF P EXTRACTED FROM THE HEN'S EGG INCUBATED FOR 18 DAYS

		Specific activity
Yolk residue	Acid-soluble P	1.56
	Phosphatide P	0.016
	Protein	0.058
Embryo	Skeleton inorg. P . . .	1.66
	Phosphatide P	1.59
	Protein	1.44

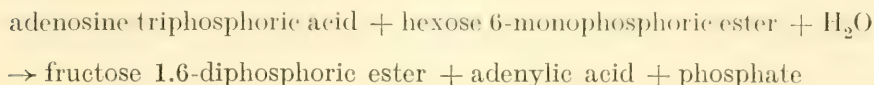
While the yolk is scarcely active, the phosphatides extracted from the embryo are found to have a very strong activity. The phosphatide molecules present in the embryo must obviously have been newly synthesized. Similar considerations apply to the protein phosphorus present in the embryo.

REACTION PATH

The path taken by organically bound phosphate radicals in glycolytic processes was investigated by using labelled compounds prepared under the action of enzymes present in muscle juice or yeast. When labelled adenyl phosphate was added to fresh muscle pulp in which glycolysis occurred, no formation of active inorganic phosphate was found to take

place, but active phosphate was detected in the HARDEN-YOUNG ester formed during alcoholic fermentation.

In a study of the interaction of labelled adenosine triphosphoric acid with non-labelled hexose monophosphoric acid ester, which leads to the formation of fructose 1.6-diphosphoric acid ester, the labelled phosphate given off by the adenosine triphosphoric acid was found to be exclusively present in the fructose 1.6-diphosphoric ester. The fact that the free phosphate formed according to the equation:



was found to be inactive, indicates that the free phosphate originated exclusively from the hexose 6-monophosphoric ester⁽⁶⁹⁾.

When both hydrogen and labelled phosphate were transferred, neither of the two stable radicals of the cozymase molecule was found to be replaced by active phosphate. A similar negative result was obtained for the reaction



in the presence of active phosphate and also for the conversion of glucose monophosphoric acid into glucose hexosephosphoric acid in the presence of active phosphate. The ester fraction were found not to have taken up ³²P.⁷⁰⁾

Does a less pronounced formation of new molecules take place simultaneously with the autolysis observed in tissue slices? By the employment of isotopic indicators this question can be answered. On shaking liver, kidney or brain slices for a few hours with a RINGER solution containing ³²P at 37° C., CHAIKOFF and his colleagues⁽⁷¹⁾ found that the phosphatides isolated from the tissue slices contained ³²P; hence, side by side with an autolysis of the phosphatides in the tissue slices, an appreciable formation of labelled phosphatides also takes place.

The formation of labelled nucleic acid in slices of JENSEN's rat sarcoma was likewise obtained⁽⁷²⁾ in the investigation of the formation of deoxyribose nucleic acid *in vitro*, when these slices were shaken with labelled blood or labelled RINGER solution. About 0.1 per cent of the deoxyribose nucleic acid molecules present in the tissue slices were found to be labelled after the lapse of 4 hours; these molecules had accordingly been formed during the experiment. The presence of cyanide, hydrogen sulphide, azide or carbon monoxide inhibits the formation of labelled phosphatides. Addition of cyanide, fluoride or moniodoacetate to labelled blood or labelled RINGER solution is also found to inhibit the formation of labelled nucleic acid.

DYNAMIC STATE OF BODY CONSTITUENTS

The most remarkable result obtained in the study of the application of isotopic indicators is perhaps the discovery of the dynamic state of the body constituents. The molecules building up the plant or animal organism are incessantly renewed. In the course of this renewal, not only the atoms and molecules taken up with the food participate, but atoms and molecules located in one organ or in one type of molecule will soon be found in another organ or in another type of molecule present in the same or in another organ. A phosphate radical taken up with the food may first participate in the phosphorylation of glucose in the intestinal mucosa, soon afterwards pass into the circulation as free phosphate, enter a red corpuscle, become incorporated with an adenosine triphosphoric acid molecule, participate in a glycolytic process going on in the corpuscle, return to the circulation, penetrate into the liver cells, participate in the formation of a phosphatide molecule, after a short interval enter the circulation in this form, penetrate into the spleen, and leave this organ after some time as a constituent of a lymphocyte. We may meet the phosphate radical again as a constituent of the plasma, from which it may find its way into the skeleton. Being incorporated in the uppermost molecular layer or into labile apatite of the skeleton, it will have a good chance to escape, but it may also have the good fortune to find a more or less lasting abode in the skeleton. This will be the case when it becomes embedded into protected, non-replaceable bone material.

There are indications that, in the growing organism, the rate of new formation of the molecules is still greater than in a fully grown organism. It was found, for example, by making use of heavy nitrogen, kindly put at our disposal by Professor UREY, as an indicator, that in "old" leaves of the sunflower, which did not develop further during the experiment, 12 per cent of the protein molecules present were renewed within 12 days. In growing leaves, the replacement of old protein molecules was found to take place at a much higher rate⁽⁷³⁾.

SCHOENHEIMER and RITTENBERG⁽³⁴⁾ have shown, by applying labelled nitrogen, that the peptide linkages in the proteins of the animal tissue are opened and reclosed with great ease. They found that the protein molecules in the living body continually change and renew their structures. This discovery is one of the most surprising and outstanding results arrived at with isotopic indicators.

EXCRETION STUDIES

Chemical analyses of the food and of the excreta permit the determination of the extent to which the organism is in balance. Chemical methods, however, cannot determine to what degree the substances found in the faeces originate from undigested food and to what extent they have been carried into the digestive tract, coming from the body proper in the form of digestive juices. This problem can be solved under strictly physiological conditions with the aid of isotopic indicators.

The simplest procedure is the following⁽⁷⁴⁾. At a suitable time after administration of labelled sodium phosphate, we determine the specific activity of the urine P and that of the faeces P. Both originate from the blood plasma and, provided that we wait for a sufficient time, the specific activity of the P compounds carried into the digestive tract from the body will be about equal to that of the urine P. If the faeces P were entirely of endogenous origin, it should show a specific activity equal to that of the urine P. If we find the faeces P to be less active than the urine P, the active faeces P of endogenous origin must have been diluted by non-active P. Since the sole source of nonactive P is the diet, the ratio of the specific activities of the faeces P and urine P tells us to what extent the endogenous faeces P has been diluted by food P.

The ratio ($100 \times$ specific activity of faeces P / specific activity of urine P) gives the percentage of P in the faeces which originates from the body proper. In the case of human subjects, $3/4$ to $4/5$ of the P present in the faeces was found to originate from non-absorbed P.

LABELLED RED CORPUSCLES

As seen in Fig. 7,⁽⁷⁵⁾ labelled phosphate penetrates at a fairly slow rate into the red corpuscles. On entering the corpuscles, however, the newly-arrived phosphate ions participate rapidly in the formation of acid-soluble organic phosphorus molecules which occur in a comparatively high concentration in the corpuscles. The formation of new acid-soluble phosphorus compounds in the corpuscles is largely associated with glycolytic processes occurring there and is attended by the destruction of an equal or almost equal number of "old" molecules. As a result of these processes, the specific activity of the labile P atoms of adenosine triphosphoric acid and that of the P of some other compounds will soon acquire a specific activity almost as high as that shown by the free P of the corpuscles, but much lower than that of the free P of the plasma. This fact and the fairly slow rate of penetration of phosphate through the corpuscle wall explain the low rate of loss of ^{32}P by labelled corpuscles when brought into contact with unlabelled plasma, and make possible

the application of such labelled corpuscles in the determination of the total circulating red corpuscle content of the organism^(76, 77). A detailed investigation of the erythrocyte content of human subjects, making use of labelled corpuscles, was carried out by NYLIN^(78, 88). He estimated,

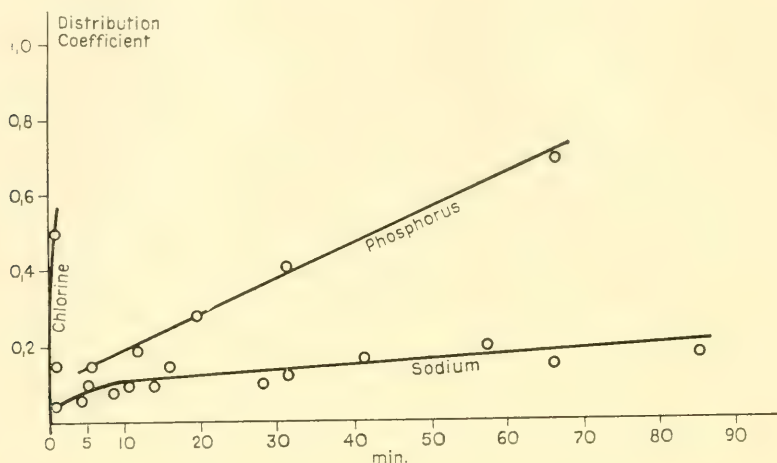


FIG. 7. — Distribution of labelled ions between corpuscles and plasma of equal weight at 37°.

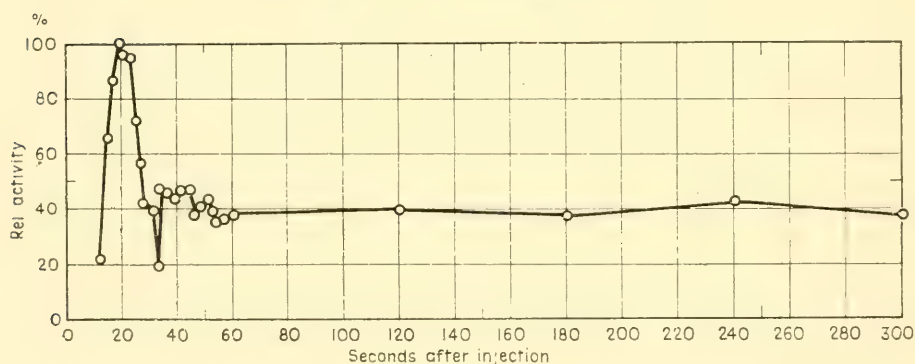


FIG. 8. — Change of activity of the arterial corpuscles with time following intravenous injection of labelled corpuscles. (G. NYLIN).

furthermore, the rate at which injected blood and the circulating blood are homogeneously mixed. Figure 8 shows the result obtained by NYLIN in an experiment where homogeneous distribution of the injected blood took only 60 seconds.

Corpuscles can also be labelled by introduction of radio-iron into the corpuscle haemoglobin. Such corpuscles were used by HAHN and his colleagues⁽⁷⁹⁾ in the determination of the red-corpuscle content of the

dog. Radio-iron has a much longer half-life period than has radio-phosphorus; such "iron-labelled" corpuscles remain labelled for a much longer time than "phosphorus-labelled" corpuscles. While, however, the latter can easily be obtained by shaking blood with labelled phosphate, the former can be made only in the living organism, a fact which, together with the relatively great difficulty of obtaining radio-iron, restricts the applicability of iron-labelled corpuscles in the determination of the erythrocyte volume.

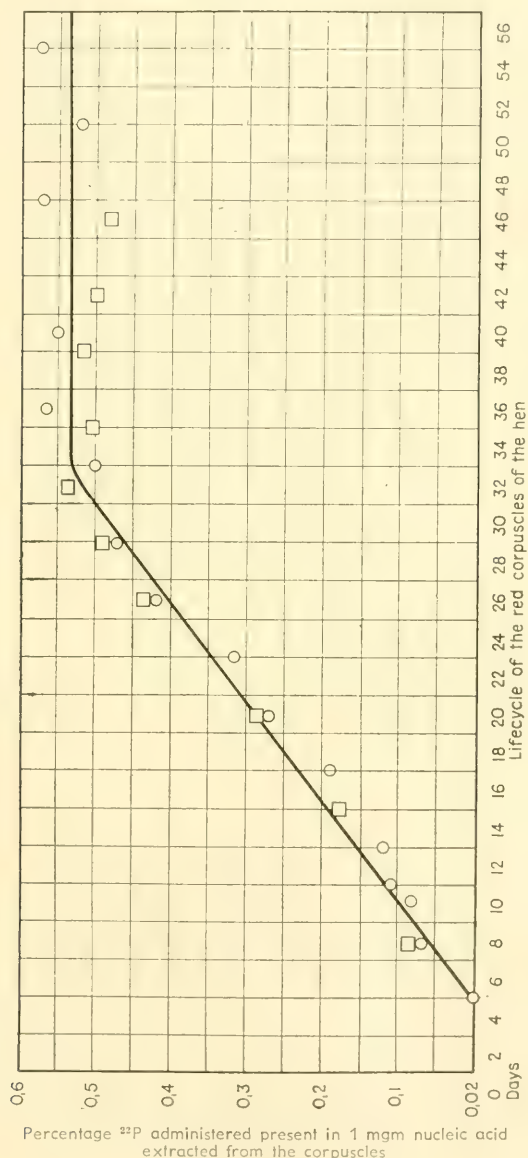


FIG. 9. — Life-cycle of the red corpuscles of the hen.

The determination of the total corpuscle volume of the organism demands only that the labelled corpuscles retain their labelling for some minutes; the determination of the life-cycle of the corpuscles requires, however, the use of marked corpuscles which conserve their labelling for weeks. No worker has yet succeeded in achieving a labelling of mammalian red corpuscles that fulfils this condition. Iron-labelled corpuscles, although remaining labelled for a sufficient time, were found by HAHN and his colleagues⁽⁸⁹⁾ not to be suited to the purpose in hand. The life of the red corpuscles of the hen, however, was determined,⁽⁸⁰⁾ making use of phosphorus-labelled corpuscles. In contradistinction to mammalian corpuscles, avian corpuscles contain large amounts of desoxyribose nucleic acid, and the nucleic acid molecules were found to remain unchanged throughout the life of the corpuscles. The newly formed corpuscles of a hen to which labelled phosphate is administered contain labelled desoxyribose nucleic acid.

By daily injection of labelled phosphate, the activity of the plasma phosphate is kept at a constant level, and at suitable intervals the specific activity of the nucleic acid P extracted from the corpuscles is determined. Figure 9 illustrates the results obtained, including the fact that, after the lapse of about 33 days, the specific activity of the nucleic acid P became constant. This indicates that all corpuscles present in the circulation of the hen were formed during the experiment. In the corpuscle samples taken in the course of the four first days, only minute amounts of labelled nucleic acid were found to be present. This may be interpreted by supposing that the formation of the corpuscles in the marrow, up to the point of their release into the circulation, requires four days. 3.5 per cent of the corpuscle content of the hen is thus built up daily.

I have attempted to give a short review of the earliest applications of isotopic indicators and to discuss a few examples of their earlier and more recent employment. Their use may be much extended in the time to come.

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99. THE APPLICATION OF RADIOACTIVE INDICATORS IN BIOCHEMISTRY

G. C. HEVESY

It is with a feeling of deep reverence that I am delivering this lecture in commemoration of the great genius and pioneer MICHAEL FARADAY.

Throughout the century which saw the birth of DALTON's atomic theory and witnessed FARADAY's fundamental discoveries, each chemical element was considered to be built up of one kind of immutable atoms. Radioactivity has completely changed this conception. As shown by RUTHERFORD and his school, we are witnessing here the decay and birth of new atomic species, and it was soon recognized that some of these, seen from the chemist's view-point, were just new editions of well-known chemical elements. To quote a few examples, it became clear that radio-thorium was chemically practically identical with thorium, and radium-D or thorium-B with lead.

The discovery of this extraordinary chemical similarity was due to SODDY, BOLTWOOD, and a few other great chemists, and the new phenomenon was termed "isotopy" by SODDY. Amongst the non-radioactive elements the existence of isotopes was first revealed in the case of neon by Sir J. J. THOMSON's study of positive rays, and then much extended by ASTON by means of his mass spectrograph. Owing to these discoveries the realm of classical chemistry was enlarged by ever-growing territories the ultimate size of which we cannot foresee. I do not venture to give even a very condensed survey of these fundamental and far-reaching advances, but shall confine myself to the discussion of the application of radioactive isotopes as indicators in biochemical studies, made possible by the above-mentioned great progress.

Radioactive isotopes were first used in the field of inorganic chemistry. The first application of this type was made at the Vienna Institute for Radium Research early in 1913 by my friend Professor PANETH, now Director of the Londonderry Laboratory of Radiochemistry in Durham, and myself. In the years to follow radioactive isotopes were applied by us, and by a small number of other workers, in various studies in the field of inorganic chemistry, and these researches proceeded at a much

enhanced rate after the discovery of artificial radioactivity and the construction of the cyclotron and the uranium pile which enlarged immensely the number of radioactive isotopes available as indicators. However, radioactive tracers found their most important application in the field of biochemistry, physiology, and pathology. The intricate chemical processes taking place in the living organism, the numerous competing routes which atoms, molecules, and larger building stones follow in the plant and animal organism, open a most fertile field for the application of radioactive isotopes.

By making use of radioactive indicators we can label atoms (ions), molecules, and even larger units such as erythrocytes, leucocytes, bacteria, and viruses; subsequently, their path and fate in the living organism can be followed. I want to consider some examples of each of these three main types of body constituents, starting with a discussion of the application of radio-sodium in the study of the distribution and circulation of sodium ions.

APPLICATION OF RADIO-SODIUM IN DISTRIBUTION STUDIES

We injected sodium chloride containing some radio-sodium (^{24}Na , half-life = 14 hours) into the circulation of the rabbit. Since the sodium content of the plasma amounts to about 2 gm, and the labelled sodium administered amounts to only a few micrograms, the change of the sodium content of the plasma caused by injection of labelled sodium remains within the limits of physiological variations. By introducing ^{24}Na we label, however, the circulating sodium ions. Within the errors of the experiments the radio-active ^{24}Na ions behave in the same manner as do the common ^{23}Na ions. The disappearance of 1% of the injected ^{24}Na from the blood plasma thus indicates the simultaneous disappearance of 1% of all sodium ions present in the circulation at the time of injection and their replacement by sodium ions present in the extravascular space.

When we first carried out such experiments in collaboration with Dr. HAHN, we were amazed at the velocity of disappearance of the individual sodium ions present in the circulation and at the velocity of interchange between intravascular and extravascular sodium, which involves a passage of the capillary wall. After the lapse of one minute almost two-thirds of the sodium ions present at the time of injection were no longer located in the circulation. The rate of disappearance of sodium from the circulation of the rabbit—more correctly the rate of replacement of plasma sodium by extravascular sodium—is seen in Fig. 1.⁽¹⁾

⁽¹⁾ HEVESY and HAHN, *Acta Physiol. Scand.* **1**, 347 (1941).

The time following the injection of labelled sodium, the injection taking a few seconds only, is plotted against the volume of diluting fluid necessary to reduce the ^{24}Na concentration of the plasma to the value observed.

Figure 1 demonstrates the fundamental difference, long known to the physiologist, between the behaviour of sodium, chloride, and bromide, on the one hand, and of potassium and phosphate, on the other. Sodium and chloride ions present at the start of the experiment not only disap-

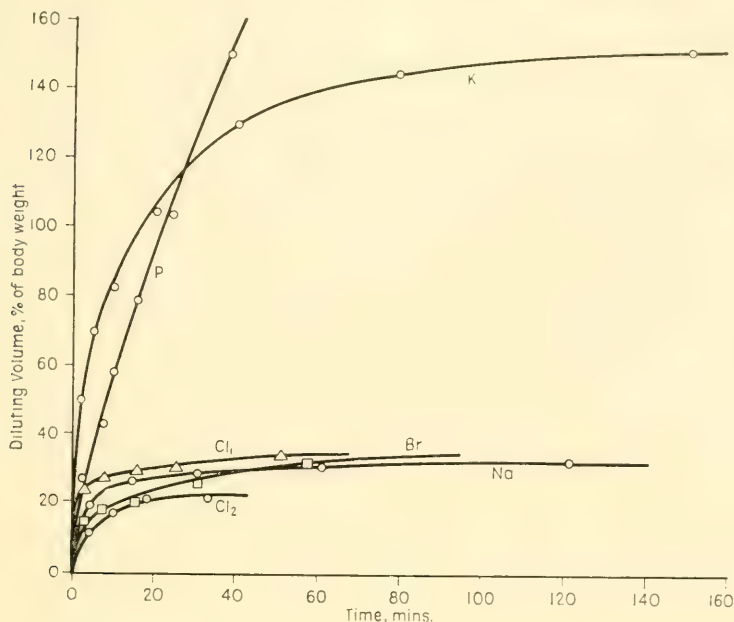


FIG. 1. — Rate of disappearance of various labelled ions from blood plasma of the rabbit (reproduced, with permission, from *Acta Physiol. Scand.* **1**, 347 (1941).

pear rapidly from the plasma, but an exchange equilibrium between the intravascular and extravascular ions is also obtained in a short time. The same is not true for potassium and phosphate. Sodium, chloride, and bromide are found mainly in the body fluid circulating outside the tissue cells, whereas potassium and phosphate appear chiefly inside the tissue cells. The intrusion of potassium or phosphate into the tissue cells requires a much longer time than the passage of the capillary wall which the "extracellular" ions alone have to perform.

FLEXNER and his colleagues,⁽²⁾ who made extensive studies on the rate of interchange of plasma and extravascular sodium, found in the

⁽²⁾ *Cold Spring Harbor Symp. Quant. Biol.* **13**, 88 (1948).

guinea-pig 60% of plasma sodium to be replaced each minute by extravascular sodium. In man, the corresponding figure was found to be 78%^(2a). Thus, in the course of each minute, about 7 gm of sodium leave the human circulation and are replaced by sodium circulating in the extravascular fluid of the various organs. This remarkable rate of interchange is made possible by the fact that 1 ml. of blood plasma in the capillaries is exposed to an area of capillary wall of 5600 cm².

By making use of radio-sodium, we can also determine the volume of the extracellular fluid present in the organism,⁽³⁾ a problem of great interest to the physiologist. From Fig. 1 it is seen that the extracellular volume indicated by the distribution of the injected sodium, the "sodium space", constitutes 23% of the rabbit's weight. By comparing the radio-sodium content of 1 g of plasma water and 1 g of fresh tissue, we find in a similar way the values for the sodium space of each organ. We can check these results by injecting into the rabbit a sodium chloride solution containing radio-chloride as an indicator.⁽⁴⁾ In both cases very similar values are obtained for the extracellular fluid volume of most organs. The "sodium space" of the skeleton, however, was found to be much larger than its "chloride space", as is seen from Table 1. From this result it follows that an appreciable part of the skeleton sodium is not circulating in the cellular interspaces, but is present as an intracellular constituent of the bone tissue, possibly replacing some bone calcium in the apatite-like mineral constituents of the skeleton.

TABLE 1. — PERCENTAGE EXTRACELLULAR WATER CONTENT OF ORGANS OF THE RABBIT, CALCULATED FROM THE RADIO-SODIUM : RADIO-CHLORIDE RATIO OF 1 GM OF FRESH TISSUE AND 1 GM OF PLASMA WATER ("SODIUM SPACE" AND "CHLORIDE SPACE")

O r g a n	Sodium space.	Chloride space.
Liver	22	25
Gastrocnemius muscle	10	9.4
Brain	10	11
Bone femur (dog)	66	24
Bone radius tibia (dog)	66	20
Bone humerus	63	18

(2a) These experiments were carried out on pregnant subjects; in normal human subjects 32% is reported (BURCH, REASER, and CRONWICK, *J. Lab. Clin. Med.* **32**, 1169 (1947).

(3) GRIFFITHS and MAEGRAITH, *Nature* **143**, 159 (1939); KALTREITER *et al.* *J. Exp. Med.* **74**, 569 (1941).

(4) MANERY and BALE, *Amer. J. Physiol.* **126**, 578 (1939).

APPLICATION OF SODIUM IN CIRCULATION STUDIES

Radio-sodium can successfully be applied in the study of the circulation velocity of the body fluids. Although, in experiments described earlier, blood-plasma samples were taken at various intervals and their activities were compared with the activity of a known aliquot of the injected sodium chloride, yet in circulation studies it may be desirable to measure outside the body the rate of propagation of the activity injected. As radio-

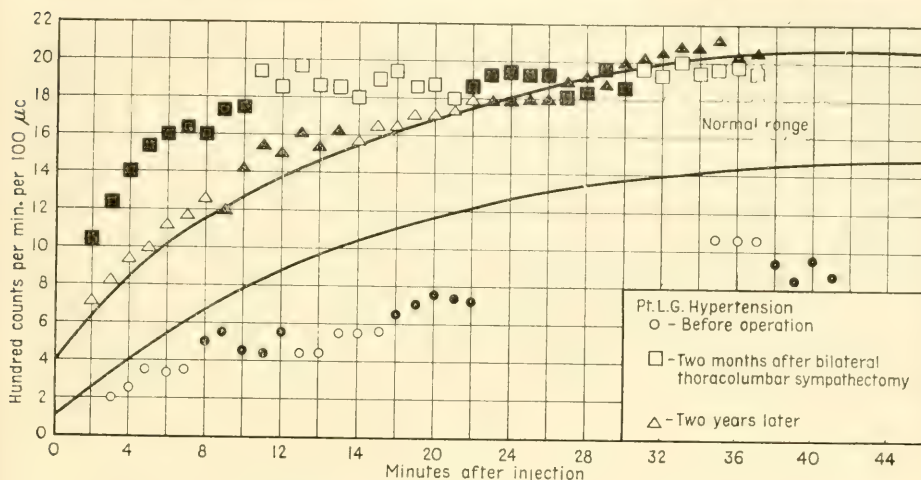


FIG. 2. — Radio-sodium "build-up" curves in a patient with severe hypertension relieved by thoracolumbar sympathectomy (Quimby) (reproduced, with permission, from *Nucleonics*, 1947, Dec., p. 1).

sodium emits not only β -rays, but also penetrating γ -rays, we can very conveniently measure the time which the injected sodium takes to reach the foot by injecting radio-sodium into the arm vein and placing a Geiger counter on the foot. The average value for the circulation time is found to be 45 seconds, the values varying between 15 and 90 seconds.

We may also be interested in determining after the injection of radio-sodium into the circulation the time necessary to establish equilibrium between the radio-sodium content of the plasma and that of the extracellular fluid. This time, although only a few minutes in the rabbit or guinea pig, is appreciably longer in human subjects; it is very different in normal subjects from that in patients suffering from peripheral vascular diseases. Such determinations may thus have diagnostic value.

QUIMBY⁽⁵⁾ injected radio-sodium into an anticubital vein and, by holding the window of a portable shielded Geiger counter against the sole of the foot, measured the arm-to-foot circulation. As long as a uni-

⁽⁵⁾ *Nucleonics*, Dec. (1947).

form distribution of the injected radio-sodium in the extracellular fluid of the body is not reached, the activity of the foot increases. In individuals with no vascular disturbance this stage is reached after the lapse of 45 minutes. Curves indicating the increase in the activity of the foot with time both in the normal and in the diseased organism are shown in Fig. 2.

For patients with various vascular disorders the curves may be within, above, or below the normal region. In the curves, solid symbols represent counts against the right foot, open ones against the left foot. The chart shows data from a patient suffering from severe hypertension relieved by bilateral thoracolumbar sympathectomy. The lowest curve (circles) represents a test made before the operation. The two upper curves; (squares and triangles) show the result of tests made two months and two years, respectively, after operation. They demonstrate the far-reaching change in the circulation of sodium ions produced by the operation.

Information on the effectiveness of blood flow can be obtained by measuring the rapidity of disappearance of radio-sodium following intramuscular injection of labelled sodium chloride⁽⁶⁾. Radio-sodium therefore proves to be an important tool in the study of circulation of body fluids, a process of basic importance.

FURTHER APPLICATIONS OF RADIO-SODIUM

The application of radio-sodium as an indicator opens a convenient way to determine the permeability of phase boundaries of different type present in the animal and plant organisms;⁽⁷⁾ nevertheless, it has its limitations.⁽⁸⁾ Radio-sodium being used as a tracer, the rate was measured at which sodium passes through the placenta from the mother to the offspring, passes from the circulation into the cerebrospinal fluid, penetrates into the aqueous tumour or through the stomach wall, intrudes into the erythrocytes,^(11, 12) is distributed between cytoplasm and nucleus,⁽⁹⁾ and so on.

Time does not permit me to describe the interesting experiments of LEVI and USSING,⁽¹⁰⁾ which prove that the two sides of the frog's skin have a distinctly different permeability to sodium. In these experiments the Ringer solution facing the inner surface of the excised frog skin was

(6) ELKIN, COOPER, ROHRER, MILLER and DENNIS, *Surg. Gynecol. Obstet.* **87**, 1 (1948).

(7) Cf. HEVESY, *Radioactive Indicators*, Interscience Publishers, New York (1948).

(8) USSING, *Cold Spring Harbor Symp. Quant. Biol.* **13**, 193 (1948).

(9) ABELSON and DURVEE, *Biol. Bull.* **96**, 205 (1949).

(10) *Nature*, Lond. **164**, 928 (1949).

labelled by addition of the short-lived ^{24}Na , while the long-lived ^{22}Na was added to the Ringer solution facing the outer skin surface.

Although the terrestrial and meteoric abundance of sodium is somewhat greater than that of potassium, yet in the living organism with very few exceptions potassium is by far the more abundant element. Mammalia, for example, contain about 3 times as much potassium as sodium. As already mentioned, potassium is located mainly in the tissue cells, sodium in the extracellular body fluid. Before the application of radioactive indicators, the great difference between the sodium content of the muscle cells and the surrounding fluid, or between the red corpuscles and the surrounding plasma, for example, was interpreted as a consequence of the impermeability of the phase boundaries to sodium. The application of radio-sodium in permeability studies revealed, however, that sodium penetrates very easily from the blood plasma into the red corpuscles, and *vice versa*⁽¹¹⁾,⁽¹²⁾ and the same applies also to potassium.

In spite of the marked permeability of the red corpuscles to sodium and potassium, the latter element is strongly (about 30 times) concentrated in the red corpuscles, while sodium is about 10 times more abundant in the plasma than in the (human) erythrocytes. Such a difference cannot be explained without assuming a distinct difference in the chemical affinity of sodium and potassium to some type of organic cellular constituents, a difference which makes potassium a more effective competitor for the cellular ionic content than sodium. LEVI and USSING⁽¹³⁾ brought important arguments for the view, formerly advocated by LUNDEGARDH⁽¹⁴⁾ and by KROGH⁽¹⁵⁾, that these hypothetical organic complexes are present in the phase boundary. The red corpuscle membrane, though impermeable to sodium ions, contains scattered anions of a substance which forms a stable complex with sodium. Owing to thermal movements these complex molecules will come into contact sometimes with the outside medium and sometimes with the inside medium. If the inside solution contains ^{24}Na ions, these may exchange with ^{23}Na in some of the complex molecules, and when these molecules later touch the outside solution, ^{24}Na will leave the complex in exchange.

The application of radioactive indicators thus revealed the existence of a new type of permeability which may be denoted as interchange permeability. It exhibits some analogy to the phenomenon of selfdiffusion. The diffusion process leads usually to changes in the concentration of the diffusing substrate: so does permeability. Self-diffusion

⁽¹¹⁾ HAHN, HEVESY and REBBE, *Biochem. J.* **33**, 1549 (1939).

⁽¹²⁾ COHN and COHN, *Proc. Soc. Exp. Biol. Med.* **41**, 445 (1939).

⁽¹³⁾ *Acta Physiol. Scand.* **16**, 232 (1948).

⁽¹⁴⁾ *Protoplasma* **35**, 548 (1941).

⁽¹⁵⁾ *Proc. Roy. Soc. B* **133**, 140 (1946).

leads to a molecular interchange only without any change of concentration. The same applies to interchange permeability. The individual sodium ions are soon found in the corpuscles and soon in the plasma without disturbing the prevailing concentration differences between the two phases.

Although by far most of the sodium of the organism is found in the circulating body fluid, yet it is not only a main constituent of that fluid but fulfils many important tasks besides this. This is obvious, *inter alia*, from the recent results obtained by BUCHANAN and his colleagues⁽¹⁶⁾ in their investigations of glycogen formation in which radio-carbon was applied as indicator. When liver slices were incubated in the presence of labelled pyruvate, formation of labelled glycogen and other labelled carbohydrates was found to take place. In these experiments the addition of sodium, but not however of potassium, to the incubating medium was found to stimulate 2- and 3-fold the formation of carbohydrates other than glycogen, while addition of potassium promoted the formation of glycogen.

LABELLING OF MOLECULAR BODY CONSTITUENTS

Rate of Molecular Renewal

In the study of the fate of molecular constituents, such as glycerophosphate, nucleic acid, or haemoglobin, in the organism we frequently do not introduce into the body labelled molecules, but marked precursors of these compounds. Introduction of glycerophosphate containing radio-phosphorus into the circulation, for example, will soon be followed by an enzymatic decomposition of that compound, a small percentage only of the glycerophosphate administered reaching the liver and other organs. It is not the determination of this percentage we are mostly interested in; we wish to know primarily the rate and the main place of formation of glycerophosphate. This information we can obtain by introducing labelled inorganic phosphate, some of which rapidly reaches the liver and is incorporated into newly formed glycerophosphate molecules. By comparing the activity of 1 mgm of liver inorganic P with that of 1 mgm of liver glycerophosphate P, we arrive at a figure characterizing the rate of formation or, as the glycerophosphate content of the liver remains constant during the experiment, the rate of renewal of glycerophosphate molecules or, more correctly, for reasons stated below, the lower limit of that rate. If after the lapse of two hours 1 mgm of glycerophosphate P has an activity corresponding to $\frac{1}{10}$ of the activity of the

⁽¹⁶⁾ BUCHANAN, HASTINGS and NESBETT, *J. Biol. Chem.* **180**, 435, 447 (1949).

inorganic P, we may conclude that $\frac{1}{10}$ of the glycerophosphate molecules present in the liver was renewed during two hours⁽¹⁷⁾. When carrying out such calculations we must take into account the change with time in the activity of 1 mgm of inorganic phosphate, usually denoted as its "specific activity". The specific activity of glycerophosphate phosphorus, for example, is the percentage of administered ^{32}P present in 1 mgm of that phosphorus. It is the specific activity of the glycerophosphate P measured at the end of the experiment which has to be compared with the mean value of the specific activity of the inorganic P during the experiment. In an experiment lasting two hours the end value and the mean value of the specific activity of the inorganic P in the liver of the rat happen to be about identical. A formula which enables us to calculate for any organ and at any time the rate of turnover from data indicating the change with time of the specific activity of both the precursor and the compound considered is given by ZILVERSMIT and his associates⁽¹⁸⁾.

The greatest difficulty we encounter when determining the renewal rates of molecular body constituents is often the lack of knowledge of the pertinent precursor of the compound. We can probably assume inorganic P to be the pertinent precursor of glycerophosphate P. In the case of lecithin, however, the labelled inorganic P may, before its incorporation into the lecithin molecules, have to be converted into glycerophosphate P or another intermediary compound. If the formation of glycerophosphate takes an appreciable time, in the first phase of the experiment, then after administration of labelled inorganic phosphate, the newly formed lecithin molecules do not become labelled as they were synthesized with the participation of yet inactive glycerophosphate molecules. When calculating the renewal rate of glycerophosphate by comparing the specific activities of the liver inorganic P and liver lecithin P we thus underestimate the turnover rate of lecithin. This would not be the case if we compared the specific activities of the glycerophosphate P and the lecithin P of the liver.

In the liver of the dog, for example, the half-life of the lecithin molecules was calculated, inorganic P being assumed to be the pertinent precursor, to be 12.5 hours, while with the assumption that glycerophosphate P is incorporated into the lecithin molecule, the much lower value of 3.6 hours was obtained⁽¹⁹⁾. These figures indicate the half-life of the average lecithin molecule of the liver. When, however, investigating the renewal rate of lecithin present in different cellular fractions of the liver, pronounced differences are found. As seen in Table 2 the

⁽¹⁷⁾ HEVESY and HAHN, *Kgl. Danske Videnskab. Selsk. Biol. Medd.* **15**, No. 5 (1940).

⁽¹⁸⁾ ZILVERSMIT, ENTENMANN and FISHLER, *J. Gen. Physiol.* **26**, 325 (1943).

⁽¹⁹⁾ ZILVERSMIT, ENTENMANN and CHAIKOFF, *J. Biol. Chem.* **176**, 193 (1948);
POPJÁK and MUIR, *Biochem. J.* **46**, 103 (1950).

rate of renewal of the phosphatide molecules present in the cell nuclei, and to a minor extent also those present in the mitochondria, is markedly lower than the corresponding figure for the average tissue phosphatides. The figures were obtained two hours after injection of labelled phosphate.⁽²⁰⁾

TABLE 2. — RENEWAL RATE OF PHOSPHATIDE P OF THE LIVER FRACTIONS OF THE RAT

Phosphatide fraction	Activity of 1 mgm of phosphatide P in percentage of the activity of 1 mgm of:		
	Plasma phosphate	Liver phosphate	Liver glycerophosphate
Total tissue	13.4	12.8	18.8
Mitochondria	9.4	9.0	13.1
Cell nuclei	5.6	5.4	7.9

Ample evidence is available to support the assumption that the incorporation of labelled phosphate into phosphatide or other organic phosphorus compounds involves enzymic processes, no "physical" interchange taking place. In the study of phosphatides in surviving liver slices incubated in a Ringer solution containing labelled phosphate, for example, the formation of "marked" phosphatides takes place under aerobic conditions only⁽²¹⁾. Absence of oxygen or presence of respiratory inhibitors should clearly not interfere with a non-enzymic type of interchange.

It was the introduction of the method of isotopic indicators which has drawn the attention to the notion of the lifetime of the molecules building up the organism, a magnitude formerly not considered. The first lifetime determination, performed almost immediately after the discovery of deuterium, was that of the water molecules of the goldfish⁽²²⁾. The water molecules present in the *living* goldfish were found to interchange rapidly with those of the surrounding water, and some of the hydrogen atoms (the "labile" hydrogen) of the organic tissue components were found to be replaced at a remarkable rate by the hydrogen atoms of the body water.

Shortly afterwards, the life-time of the water molecules present in the human body was measured^(22a). Dilute heavy water being used as an indicator, following the intake of a known volume of dilute (0.5 molar %)

⁽²⁰⁾ DE ELLIOTT and HEVESY, *Acta Physiol. Scand.* **19**, 370 (1950); cf. Ada, *Biochem. J.* **45**, 422 (1949).

⁽²¹⁾ TAUROG, CHAIKOFF and PERLMAN, *J. Biol. Chem.* **145**, 281 (1942).

⁽²²⁾ HEVESY and HOFER, *Z. physiol. Chem.* **225**, 28 (1933).

^(22a) *Idem*, *Nature* **134**, 879 (1934).

heavy water, the heavy water content of the urine was determined; from these and other excretion figures the average life-time of water molecules in a test person was found to be 14 days. This value clearly depends not only on the total water content but also on the water intake of the subject.

In the same investigation the total water content was calculated from the heavy water content of the water drunk and that of the body water after exchange equilibrium between the water molecules taken in and those already present in the organism was reached. The heavy water content of the body water was obtained by determining the heavy water content of urine water. The total water content of the test person was found to form 64% of the body weight. For abnormally adipose individuals figures as low as 40%, for lean ones as high as 70%, have recently been obtained⁽²³⁾.

The interest of the biochemist in the life-time of the molecules building up the organism was soon much enhanced by the classical work of SCHOENHEIMER and RITTENBERG⁽²⁴⁾, who determined first the lifetime of fat molecules and later many other types of molecules in the animal body and, moreover, by the investigation of radio-phosphorus in similar investigations, the first of which was the renewal rate of the mineral constituents of the bone.⁽²⁵⁾

Place of Formation of Molecular Constituents

Having discussed the rate of renewal of phosphatide molecules, I want now to say a few words about the place of formation of phosphatide molecules in the yolk of the hen's egg and in the blood plasma. The determination of the place of formation of body constituents is another important field of application for isotopic indicators.

Only a few hours after administration of labelled phosphate to the hen, the presence of radioactive lecithin and other phosphatides can be detected in the yolks of the ovary. These phosphatide molecules may have been synthesized in the yolk or carried into the yolk by the circulating plasma, an alternative explanation being the incorporation into the yolk of phosphatide molecules built up in the ovary. Now, it can be shown by experiments *in vitro* that penetration of labelled phosphates into the egg followed by intrusion into the yolk does not lead to the formation of labelled phosphatide molecules⁽²⁶⁾.

⁽²³⁾ DRABKIN, *Fed. Proc.* **9**, 182 (1950).

⁽²⁴⁾ J. Biol. Chem. **111**, 175 (1935); SCHOENHEIMER, *The Dynamic State of Body Constituents*, Cambridge, Mass. (1942).

⁽²⁵⁾ CHIEVITZ and HEVESY, *Nature*, Lond. **136**, 753 (1935).

⁽²⁶⁾ HEVESY and HAHN, *Kgl. Danske Videnskab. Selsk. Biol. Medd.* **14**, No. 2 (1938).

In view of the fact that the specific activity of the phosphatides of the ovary is lower than that of the yolk phosphatides, as seen in Table 3, the former cannot be the source of the latter⁽²⁶⁾.

TABLE 3. — SPECIFIC ACTIVITY OF PHOSPHATIDE P
IN ORGANS OF THE HEN

Organ	Relative specific activity
Liver	100
Plasma	79
Ovary	7.2
Yolk	9.2
Intestinal mucosa	18

The specific activity of the precursor of a product obviously cannot be lower than that of the product itself, at least at that phase of the experiment in which the specific activity of the precursor increases with time. Thus we have to conclude that the yolk phosphatides originate from the plasma phosphatides. The source of the plasma phosphatides, or at least their main source, must be the liver phosphatides as alone the liver phosphatides have a higher specific activity than the plasma phosphatides. Even the specific activity of the phosphatides of the intestinal mucosa in which phosphatides are turned over at a remarkable rate as seen in Table 3 is lower than that of the plasma phosphatides.

While the plasma phosphatides contain mainly lecithin, appreciable amounts of cephalin are also present in the liver. In view of the small differences in the turnover rate of lecithin and cephalin in the liver, the difference in the composition of liver and plasma phosphatides does not influence the above conclusion.

The most direct approach to the solution of the problem of the origin of plasma phosphatides is a comparison of the specific activity of plasma phosphatides in the intact and in the hepatectomized animal. In their experiments FISHLER *et al.*⁽²⁷⁾ found that, after the administration of labelled phosphate in contrast to the phosphatides of the plasma of the intact dog, the plasma phosphatides of the liver-less dog contained only a negligible amount of ³²P. As in both cases the same amount of labelled phosphate was administered to the dog, this result fully confirms the above conclusion that the liver is almost the sole place of formation of plasma phosphatides⁽²⁸⁾.

⁽²⁷⁾ FISHLER, ENTENMANN, MONTGOMERY and CHAIKOFF, *J. Biol. Chem.* **150**, 47 (1943); ENTENMANN, CHAIKOFF and ZILVERSMIT, *Ibid.* **166**, 15 (1946).

⁽²⁸⁾ Cf. also POPJÁK and BEECKMANS, *Biochem. J.* **46**, 99 (1950).

The above-mentioned lack of extra-ovarian formation of labelled phosphatide in the yolk is also brought out in experiments with incubated fertilized eggs into which labelled phosphate was injected⁽²⁹⁾. While the foetal phosphatides present in such eggs have a high ^{32}P content, the remainders of the yolk still present in such eggs do not contain significant amounts of labelled phosphatides, as seen in Table 4. The slight activity of yolk phosphatides, which increases with age of the embryo, is possibly caused by influx from the embryo into the yolk of a small amount of labelled phosphatides or of the enzymes responsible for resynthesis of phosphatides.

TABLE 4. — SPECIFIC ACTIVITY OF PHOSPHATIDES EXTRACTED FROM EMBRYO AND RESIDUAL YOLK OF THE HEN'S EGG

Time of incubation days	Phosphatides extracted	Specific activity
6	Yolk	0.032
	Embryo	100
11	Yolk	0.10
	Embryo	100
18	Yolk	0.92
	Embryo	100

Similar considerations were applied by ATEN⁽³⁰⁾ to the study of the origin of the milk phosphatides in the goat. Table 5 demonstrates that at least most of the phosphatide molecules of the milk are not those which passed from the blood plasma into the milk, but those which had their origin in the milk gland, the phosphatides of the milk being much more active than those of the plasma, but less active than those of the milk gland.

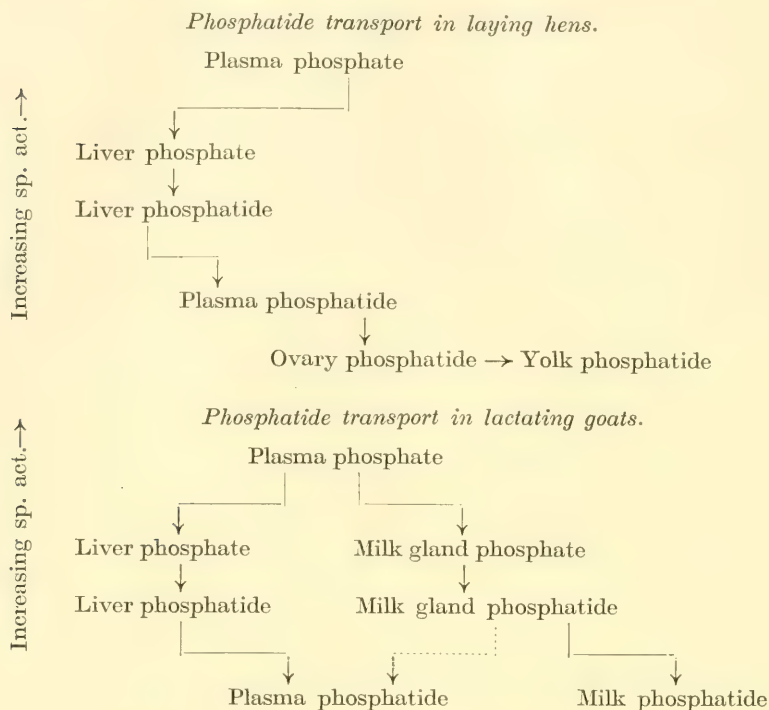
The following scheme represents the course of phosphatide transport in laying hens and in lactating goats⁽³⁰⁾.

TABLE 5. — ACTIVITY OF PHOSPHATIDE PHOSPHORUS OF MILK AND ORGANS OF A GOAT³⁰

Organ	Specific activity	Organ	Specific activity
Milk	1	Liver	1
Plasma	0.02	Kidneys	1.2
Milk gland	1.4		

⁽²⁹⁾ HEVESY, LEVI and REBBE, *Ibid.* **32**, 2147 (1938).

⁽³⁰⁾ ATEN, *Isotopes and Formation of Milk and Egg*. Diss., Utrecht (1939); ATEN and HEVESY, *Nature* **142**, 111 (1938).

Scheme for the specific activity of phosphatides²⁹**Effect of Irradiation with X-Rays on the Formation of Nucleic Acids**

The application of radioactive indicators proved to be an efficient tool for the measurement of the change in the rate of formation of molecular body constituents caused by administration of chemicals inducing such interference or by irradiation with ionizing radiation which produces such interfering products *in situ*. Such radiation may diminish the growth rate, and we can expect that it might interfere with the rate of formation of some cellular constituents already in the early or earliest phase of the experiment and with that of deoxyribonucleic acid, a main constituent of the cell nucleus.

In a growing tumour, growth, and correspondingly, additional formation of deoxyribonucleic acid, may amount to about 1% in the course of 2 hours. If this formation is suppressed by irradiation, the irradiated tumour after the lapse of 2 hours can be expected to contain 1% less deoxyribonucleic acid than do the controls. The distinction between 100 and 99 deoxyribonucleic acid molecules present in a tumour by means of chemical analyses is a most difficult task. However, when applying radioactive indicators such a difference can easily be measured. Radioactive indicators permit us to distinguish between old and new molecules, between those present before the start of the experiment and those

formed afterwards. The old molecules are not radioactive, but the new ones contain ^{32}P .

Let us assume that out of 100 deoxyribonucleic acid molecules present in the non-irradiated tumour two are found to contain ^{32}P two hours after administration of labelled phosphate while in the irradiated tumour, in which deoxyribonucleic acid formation is suppressed, the corresponding figure is only 1. Such a difference is readily ascertained. In the first case, the Geiger counter or another suitable measuring instrument will indicate an activity of, say, 200 counts per minute, in the second case only 100. Radioactive measurements can very easily be made, a fact which contributes materially to the wide application of the method of radioactive indicators.

In Table 6 the effect of irradiation with X-ray doses ranging from 335 to 1500 r. on the Jensen-sarcoma of the rat is shown^(30a). A few minutes after the irradiation, labelled sodium phosphate was injected into 68 rats and into the same number of controls. The effect of irradiation on the formation of deoxyribonucleic acid molecules is seen to be most effective shortly after irradiation.

TABLE 6. — EFFECT OF X-RAYS ON THE FORMATION OF LABELLED DEOXYRIBONUCLEIC ACID AFTER ADMINISTRATION OF LABELLED SODIUM PHOSPHATE

Dose, in r units	Duration of experiment, hours	Ratio of labelled nucleic acid formation in non-irradiated and irradiated sarcoma
750—1500	$\frac{1}{2}$	3.2
350—1500	1	2.4
650—1500	2	2.2

In another set of experiments⁽³¹⁾ with 32 rats the mean ratio of the labelled nucleic acid formation in the non-irradiated and irradiated rats after the lapse of 2 hours was found to be 2.05, and in a third set⁽³²⁾ (40 animals) 2.2. Similar results were obtained by BARBARA HOLMES⁽³³⁾, who, injecting labelled phosphate into 14 rats, found a mean value of 2.1 for the above ratio $\frac{1}{2}$ —2 hours after irradiation with 2000 r.

In organs such as liver, kidney, spleen, and intestinal mucosa, the formation of deoxyribonucleic acid formation was found to be reduced

^(30a) VON EULER and HEVESY, *Arkiv Kemi* **17**, A Nr. 30 (1944); HEVESY, *Rev. Mod. Physics* **17**, 102 (1950).

⁽³¹⁾ VON EULER and HEVESY, *Kgl. Danske Videnskab, Selsk. Biol. Medd.* **17**, Nr. 8 (1942).

⁽³²⁾ AHLSTRÖM, V. EULER and HEVESY, *Arkiv Kemi* **19**, A Nr. 13 (1945).

⁽³³⁾ *Brit. Rad. J.* **20**, 45 (1947); **22**, 487 (1949).

to a similar extent to that in the tumour⁽³⁴⁾ under the effect of ionizing radiation. HARDIN JONES's results⁽³⁵⁾ in this field are instructive. He compared the depressing effect of whole-body radiation on blood-cell counts with the reduction in the turnover rate of formation of labelled deoxyribonucleic acid in tumour and liver. Some of his results are seen in Table 7. They show similar figures for percentage depression of blood corpuscles, which is at least partly caused by inhibition of deoxyribonucleic acid formation in the bone marrow, and deoxyribonucleic acid formation in the tumour and liver.

TABLE 7. — DEPRESSING EFFECT OF IRRADIATION WITH A DOSE OF 1 R. OF X-RAYS (HARDIN JONES)

System investigated	Percentage depression due to irradiation
White corpuscle count or lymphocyte count	0.23
Red corpuscle count	0.3
Formation of labelled deoxyribonucleic acid in tumour	0.18
Formation of labelled deoxyribonucleic acid in liver	0.28

INDIRECT RADIATION EFFECTS

The work of DALE⁽³⁶⁾ and others on the effect of X-rays on enzyme suspensions led to the result that part of the effect of irradiation on enzymes is caused by the production of noxious agents in the solvent. Inactivation of the enzymes is to a large extent caused by the interaction of these noxious agents produced in the water with the enzyme. DALE found that with a given dose inactivation is obtained of a considerably smaller fraction of a concentrated than of a dilute carboxy-peptidase solution. This observation suggests the explanation, and the only feasible one, that the number of inactivating atoms or molecules produced under the effect of weak doses of X-rays in the solvent suffices only to inactivate a restricted number of enzyme particles. The primary process in the action of radiation on water is detachment of an electron and its subsequent transfer from the ion to one of the neighbouring molecules or ions⁽³⁷⁾. For pure water the primary process is $(\text{HO})^-\text{H}^+ + \text{radiation} \rightleftharpoons \text{HO} + \text{H}$.

Most of these decomposition products have an exceedingly short life and may recombine to form water or react with protective molecules present before they reach the enzyme surface. Besides the existence of

⁽³⁴⁾ AHLSTRÖM, EULER and HEVESY, *Arkiv Kemi* **19**, A Nr. 9 (1944).

⁽³⁵⁾ *Proc. Oberlin College Radiation Biology Meeting*, in press.

⁽³⁶⁾ *Biochem. J.* **34**, 1367 (1940).

⁽³⁷⁾ WEISS, *Nature* **53**, 748 (1944).

this indirect radiation effect due to more or less short-lived radicals, the application of labelled phosphate in radiation studies revealed the existence of another type of indirect effect⁽³⁸⁾ possibly due to products of radiation.

In animals bearing two distant tumours, one of which was irradiated while the other was effectively shielded with lead, incorporation of ^{32}P into the deoxyribonucleic acid of not only the irradiated but also the shielded sarcoma was observed. Irradiation with a dose of 280–2000 r had an effect on the deoxyribonucleic acid formation of the shielded tumour which lagged behind with only 20–40% of that observed in the irradiated sarcoma.

KELLY and HARDIN JONES⁽³⁹⁾ extended these studies and found that even local irradiation of the liver of the rat with a dose of 170 r leads to a 34% reduction of labelled deoxyribonucleic acid formation in the tumour, while local irradiation of the muscles with 230 r reduces the labelled deoxyribonucleic acid formation in the liver by 26%.

On introduction of the blood of a strongly irradiated rabbit into the circulation of another rabbit, the rate of incorporation of ^{32}P into deoxyribonucleic acid of the kidneys of the second rabbit was found to be reduced, an observation which suggests the presence of a substance in the circulation of the irradiated rabbit which has a blocking effect on nucleic acid formation⁽⁴⁰⁾. In view of more recent results obtained when using ^{14}C as an indicator in radiation studies, which I shall discuss later, such indirect effects appear less puzzling now than at the time of their observation.

The availability of the long-lived (half-life = 5700 years) ^{14}C for tracer research was an event of great importance. It immensely increased the stimulus already provided by the applicability of the short-lived ^{14}C (half-life = 20 minutes) and the stable ^{13}C . To mention only a single example, it elucidated to a remarkable extent many steps involved in glycogen synthesis. ^{14}C was also applied in the study of the biochemical effects of X-rays. Into a great number of rapidly growing rats, ^{14}C incorporated with the carboxyl group of sodium acetate was injected. Before the injection, half of the rats were irradiated with a dose of 900 r. The silver purines of deoxyribonucleic acid isolated from some of the organs were then obtained, and their radioactivity was compared⁽⁴¹⁾. On irradiating new-born mice with about 900 r, the incorporation of ^{14}C into the purines of the deoxyribonucleic acid was found to be depressed

⁽³⁸⁾ AHLSTRÖM, V. EULER and HEVESY, *Arkiv Kemi* **19**, A Nr. 13 (1945); HOLMES, *Brit. J. Radiol.* **22**, 487 (1949).

⁽³⁹⁾ *Proc. Soc. Exp. Biol. Med.* **74**, 493 (1950).

⁽⁴⁰⁾ AHLSTRÖM, V. EULER, HEVESY and K. ZERAHN, *Arkiv Kemi* **23**, A Nr. 11 (1946).

⁽⁴¹⁾ HEVESY, *Nature* **163**, 869 (1949).

to an extent similar to that of ^{32}P incorporation into deoxyribonucleic acid in the above-mentioned experiments. Furthermore, experiments carried out in Chicago^(41a) brought out a depressing effect of irradiation on the incorporation of ^{14}C into the purines of ribonucleic acid similar to that observed in the case of the deoxyribo-compound. The incorporation of ^{32}P into ribonucleic acid⁽³³⁾ was found, however, to be markedly less susceptible to the effect of irradiation than its incorporation into the deoxy-compound.

RENEWAL OF RIBONUCLEIC ACID

A very appreciable part of the incorporation of ^{32}P into ribonucleic acid molecules may be due to rephosphorylation of such molecules. If, in contrast to the synthesis of the total molecule the rephosphorylation process is not radiosensitive, we may find an explanation in the difference of the effect of radiation on the incorporation of ^{32}P and ^{14}C into the ribonucleic acid molecules. This explanation is very hypothetical, and I mention it mainly because it offers a welcome opportunity to emphasize the fact that isotopic tracers may indicate fundamentally different processes, such as renewal of one of the molecular constituents only or synthesis of the whole molecule from primitive precursors and, furthermore, intermediates between these two extreme cases.

Let us consider a compound such as adenosine triphosphoric acid. This molecule contains two labile, easily removed phosphate groups, and a third one incorporated into the adenylic acid moiety. In the animal organism, intrusion of the administered labelled inorganic phosphate into tissue cells is followed by a strikingly rapid interchange between the labelled phosphate ions and the labile phosphate groups of the adenosine triphosphoric acid. An interchange may even take place during the passage of the labelled inorganic phosphate through the boundary of the intra- and extra-cellular spaces, as pointed out by Sachs⁽⁴²⁾. The adenylic acid moiety is not involved in this very rapid renewal process. The rate of incorporation of ^{32}P into the adenosine triphosphate is thus a measure of the rate of rephosphorylation only of that molecule and not of its formation from early precursors. Even in a rapidly growing mammalian organ, in which the adenosine triphosphoric acid content may increase by as much as 1% per hour, such an increase involving 1% additional formation of adenosine triphosphoric acid from early precursors, the incorporation of ^{32}P is negligible compared to incorporation of ^{32}P by rephosphorylation.

(41a) Personal communication by Dr. GUZMAN BARRON.

(42) *Cold Spring Harbor Symp. Quant. Biol.* **13**, 180 (1948).

Incorporation of ^{32}P into deoxyribonucleic acid of a growing organ, e.g. a tumour, is of a very different type. Even if some rephosphorylation in such molecules cannot be excluded, the incorporation of ^{32}P into deoxyribonucleic acid of a growing organ takes place mainly in the course of mitotic processes in which necessarily the whole molecule is involved. This appears from the parallelism often found between the mitotic figure and the rate of ^{32}P incorporation into deoxyribonucleic acid of the organ. In organs in which appreciable cell division takes place, such as in the bone marrow, the thymus gland, the intestinal mucosa, or the spleen, administration of labelled phosphate to the rat is promptly followed by a remarkable formation of labelled deoxyribonucleic acid molecules. This is not the case in the liver or kidney of the fully-grown animal in contrast to the corresponding organs of the newly-born rat. We shall see later, when discussing the life-cycle determination of blood corpuscles, that the deoxyribonucleic acid phosphorus of the circulating avian red corpuscle and of the mammalian white corpuscle in which mitotic processes do not occur is entirely stable during the life-time of such particles, and that incorporation of ^{32}P into the deoxyribonucleic acid of such particles takes place only during their formation. The Jensen-sarcoma of the rat grows by about 1% per hour, and its deoxyribonucleic acid content increases correspondingly by about 1%.

In the Jensen-sarcoma the probability of the incorporation of a ^{32}P atom into a ribonucleic acid molecule was found to be 2–3 times higher than that of its incorporation into a deoxyribonucleic acid molecule⁽⁴³⁾. As during the 2-hour experiment the percentage additional formation of ribonucleic acid in the growing tumour cannot differ much from that of deoxyribonucleic acid, the above figures indicate that out of three ^{32}P atoms at least 1–2 are incorporated into ribonucleic acid by a process which does not involve synthesis of ribonucleic acid molecules from early precursors.

TABLE 8.—EFFECT OF IRRADIATION WITH AN X-RAY DOSE OF 880 R ON THE INCORPORATION OF ^{14}C INTO DEOXYRIBONUCLEIC ACID PURINES AND INTO TISSUE PROTEINS PREPARED FROM THE LIVERS OF 55 NEW-BORN FED RATS (MEAN VALUES)

Fraction	Percentage change in the incorporation of ^{14}C due to the effect of irradiation
Purines	—44
Proteins	+27

⁽⁴³⁾ EULER, HEVESY and SOLODKOWSKA, *Arkiv Kemi* **26**, A Nr. 4. (1948).

In experiments in which the effect of irradiation on the incorporation of ^{14}C into the deoxyribonucleic acid was studied, acetate labelled with ^{14}C in the carboxyl group was injected into mice. In Table 8, in addition to the effect of irradiation on ^{14}C incorporation into purine carbon of deoxyribonucleic acid, the corresponding effect on ^{14}C incorporation into protein of the liver is seen as well. Whereas incorporation into purines is markedly depressed, that into proteins is enhanced under the effect of irradiation.

CHANGE OF SENSITIVITY OF THE RADIOACTIVE INDICATOR IN THE COURSE OF THE EXPERIMENT

When applying isotopic indicators in the study of animal metabolism, two main lines of technique can be followed. We can keep the tracer in the body at a constant level. Deuterium for example, being used as an indicator, dilute heavy water is given to the animal throughout an experiment taking days or weeks. The body fluids soon reach a constant heavy-water content and, by comparing the deuterium content of the body water with that of fatty acids extracted from an organ, we can state what percentage of the fatty acid deuterium of that organ reached the body-water deuterium level and thus find the percentage fatty acid renewal during the experiment or, more correctly, the lower limit of that percentage. This method was much applied by SCHOENHEIMER and RITTENBERG in their classical experiments and also by STETTEN, BLOCH, BERNHARD, and others, who obtained important information⁽²⁴⁾.

When studying acetate metabolism, acetate labelled, for example, in the carboxyl group with ^{13}C or ^{14}C can be added to the daily food and an almost constant ^{13}C or ^{14}C content of the acetate of the body fluid will thus be obtained. As shown by RITTENBERG and BLOCH, and also by others^(43a), here again a comparison of the ^{13}C or ^{14}C content of the ingested acetate with that of fatty acid or cholesterol, etc., yields the lower limit of percentage renewal of these compounds.

In the work with radioactive indicators we mostly choose, however, a different technique. We administer the labelled precursor at the start of the experiment only, so the specific activity of the indicator decreases with time. On injection of labelled phosphate of negligible weight, having an activity of 1 mc., into the circulation of a human subject containing 130 mgm. of plasma inorganic P this mc. will first indicate the presence of 130 mgm. of inorganic phosphorus. Soon, however, as the ^{32}P of the plasma inorganic P rapidly passes the capillary wall, it inter-

(43a) RITTENBERG and BLOCH, *J. Biol. Chem.* **160**, 417 (1946); PIHL, BLOCH and ANKER, *Ibid.* **183**, 441 (1950).

changes with the extra-vascular inorganic ^{31}P . Owing to these and other interchanges, the ^{32}P content of the blood plasma rapidly decreases. After the lapse of 60 minutes, the ^{32}P content is reduced to about one-tenth of its original value, the presence of 130 mgm of inorganic P in the blood plasma being now indicated by an activity of 0.1 mc. only. The sensitivity of the radioactive indicator is thus increased to 10 times its initial value. A similar behaviour is shown by many other radioactive indicators.

The sensitivity of acetate ^{14}C as an indicator of body acetate increases at a remarkable rate, as seen in Fig. 3.⁽⁵⁶⁾ In this figure are plotted the

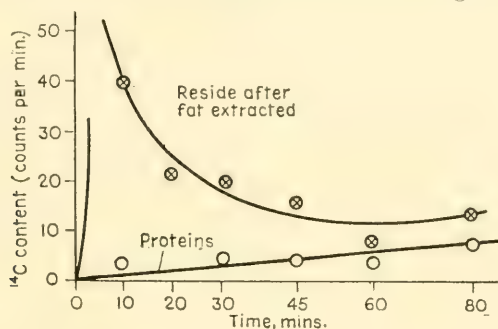


FIG. 3. — Change with time in the ^{14}C content of brain fractions of mice after intraperitoneal injection of labelled acetate (reproduced, with permission, from *Arch. internat. Pharm. Therap.* **86**, 33 (1943).

results of experiments in which groups of mice were killed at different times after interperitoneal injection of acetate labelled in the carboxyl group with ^{14}C . The radioactivity of the fat-free brain tissue and also that of the proteins of the brain was then determined, and the figures obtained plotted against time. After only 30 minutes, the activity of the fat-free (acid-soluble + protein) fractions is less than half of the value observed after 10 minutes, the protein fractions showing a slow increase in their ^{14}C content with time.

The change of sensitivity of the radioactive indicator with time may be disturbing and can make the interpretation of the results obtained very difficult. On the other hand, it may also have great advantages. We shall describe some examples showing both disadvantages and advantages which result from the marked time-dependency of the sensitivity of radioactive indicators.

RENEWAL OF THE MINERAL CONSTITUENTS OF THE SKELETON

The great inconvenience inherent in the change of the sensitivity of radioactive indicators with time becomes clear from an attempt to determine the rate of renewal of the mineral constituents of the skeleton by

making use of radio-phosphorus.⁽⁴⁴⁾ Here we refer to the first turnover studies of body constituents with the help of a radioactive tracer, which were carried out simultaneously with the above-mentioned first investigations by SCHOENHEIMER and RITTENBERG, who used deuterium as an indicator.

Blood-plasma phosphate was found to interchange very rapidly with the uppermost phosphate layer of the apatite-like crystallites. This rapid interchange is followed by a slower one due to a slow recrystallization

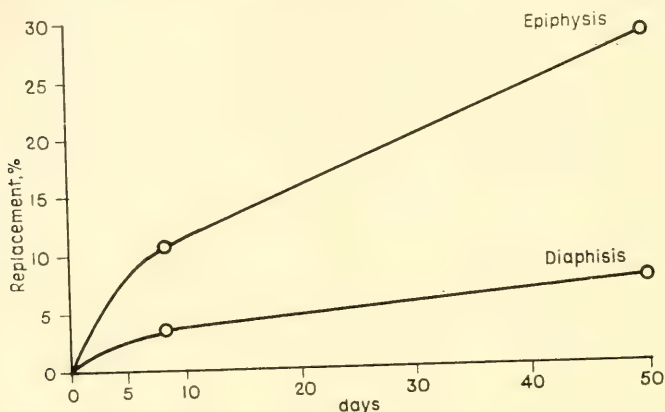


FIG. 4. — Extent of replacement of rabbit's bone phosphorus by labelled phosphorus (reproduced, with permission, from *Biochem. J.* **34**, 532 (1940))

of the apatite crystals. Some molecular layers go into solution while others are formed with the participation of labelled phosphate. Such layers will be comparatively strongly active in the early phase of the experiment. Owing to the decrease in the ^{32}P content of the plasma and lymph with time, they will be much less active in later phases.

On top of a strongly active layer, slightly active layers may be deposited, protecting the first layer from dissolution. The great complexity of ^{32}P distribution in the skeleton apatite frustrates, or at least makes extremely difficult, a quantitative determination of the extent of renewal of the mineral constituents of the bone when labelled phosphate is administered only at the start of the experiment. We arrive at such a result when administering daily repetitions of labelled phosphate, thus keeping the level of plasma activity constant. By applying this technique, which is much less convenient, and by comparing the specific activities of the bone inorganic P and plasma inorganic P at the end of the experiment, we obtain a figure indicating the percentage of the renewed skeleton. Results obtained with rabbits, seen in Fig. 4, show that after the lapse

⁽⁴⁴⁾ CHIEVITZ and HEVESY, *Nature* **136**, 754 (1935).

of 50 days 70% of the soft bones (epiphysis) and as much as 93% of the hard bones (diaphysis) remained unchanged⁽⁴⁵⁾. No similar experiments were carried out with human subjects but, as the extent of renewal increases only slightly with increasing time, we seem to be justified in concluding that a large part of the mineral constituents of the adult skeleton remains unchanged during life.

The above-mentioned protection of highly active phosphate apatite layers by slightly active ones much resembles the protection of lead incorporated with the skeleton. From a blood plasma with comparatively high lead content, lead-replacing calcium ions are incorporated with an apatite layer. Since the lead content of the plasma decreases with time, the above-mentioned layer may be covered by others containing only negligible amounts of lead. The probability of an escape of lead from the skeleton is thus strongly reduced. It takes place, however, to some extent over long time intervals, constantly supplying the plasma with toxic lead. Not only lead but also numerous other elements, e.g. uranium,⁽⁴⁶⁾ find more or less permanent abode in the skeleton.

DETERMINATION OF THE LIFE-CYCLE OF BLOOD CORPUSCLES

In the determination of the life-time of blood corpuscles, the marked decline of the specific activity of the precursor with time proves to be most advantageous, as shown, for example, in OTTESEN's work^(46a) on the life-cycle of nucleated red corpuscles. After administration of labelled phosphate, the specific activity of the inorganic phosphate of the blood plasma declines, as seen in Fig. 5. It takes some time for the inorganic phosphate of the marrow to come into exchange equilibrium with the inorganic phosphate of the plasma, but after that, the specific activity of the marrow inorganic phosphate will markedly decrease with time as well. Thus, the deoxyribonucleic acid molecules formed with participation of labelled inorganic phosphate in the course of the first day are much more active than those formed later. The deoxyribonucleic acid-containing red corpuscles have a definite life-time. As soon as this is reached they are destroyed and their nucleic acid phosphorus, which is now exposed to the effect of phosphatase, is split off and soon lost in the larger pool of inorganic phosphate present in the body. The end of the life-cycle of the red corpuscles will thus be indicated by a sudden decrease in the activity of the deoxyribonucleic acid extracted from the erythrocytes.

⁽⁴⁵⁾ HEVESY, LEVI and REBBE, *Biochem. J.* **34**, 532 (1940).

⁽⁴⁶⁾ NEUMAN and NEUMAN, *J. Biol. Chem.* **174**, 711 (1948).

^(46a) *Nature* **162**, 730 (1948); Diss., Copenhagen (1951).

(a) Life-time of the Avian Red Corpuscles

In the determination of the life-cycle of avian red corpuscles, erythrocyte samples are secured daily after injection of labelled sodium phosphate (about $15 \mu\text{c.}$) to the hen, and the radioactivity of their deoxyribonucleic acid P is determined. The specific activity of the plasma inorganic P is determined as well. In Fig. 5 the broken line indicates the change of the specific activity of plasma inorganic P with time, and the full line shows the corresponding values of deoxyribonucleic acid P.

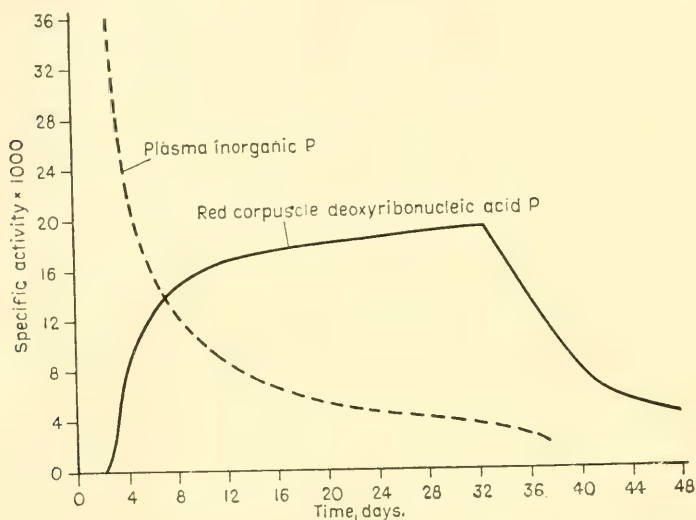


FIG. 5. — Change of the specific activity of phosphorus fractions of the hen's blood with time (OTTESEN).

The radioactivity of the deoxyribonucleic acid P of the red corpuscles formed during the first day constitutes a very large part of the radioactivity of the deoxyribonucleic acid of the total red corpuscle content of the circulation. Correspondingly, as soon as the life-cycle of the red corpuscles formed during the first day after injection of labelled sodium phosphate is accomplished, the activity of the deoxyribonucleic acid P of the total corpuscles rapidly declines, as seen in Fig. 5. The date of this rapid decline indicates almost precisely the life-cycle of the avian red corpuscles.

To arrive at a correct value of the life-cycle of the deoxyribonucleic acid, the contribution of red corpuscles formed in a later part of the experiment to the activity of the total erythrocyte deoxyribonucleic acid must be taken into account as well. This can be done by making use of the following consideration. The formation of labelled deoxyribonucleic acid during the first day takes place with participation of

inorganic P the specific activity of which corresponds to the mean value of the inorganic P during that time interval. This mean value, which can be experimentally determined, is denoted by $f(\frac{1}{2})$. The contribution of active deoxyribonucleic acid of red corpuscles formed during the first day of the experiment to the total activity of erythrocyte deoxyribonucleic acid measured after the lapse of 1 day is $f(\frac{1}{2}) (\Phi \frac{1}{2})$, where Φ is a function of the time taken by the transition of the red corpuscles from the marrow into the circulation, and also of the duration of the life-cycle of the erythrocytes. Actually, we compare, not the deoxyribonucleic acid activities of the total erythrocyte content of the circulation, but the specific activities of deoxyribonucleic acid P fractions.

The second day, the corpuscle deoxyribonucleic acid is formed from inorganic P having a much lower specific activity than the first day

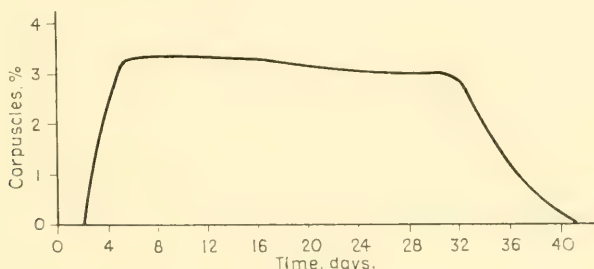


FIG. 6. — Percentage of hen red corpuscles, formed during the first day of the experiment, present in blood stream at the time indicated on the x-axis (OTTESEN).

the mean value of which is now $f(\frac{1}{2})$. The contribution of active deoxyribonucleic acid formed during the second day to the total deoxyribonucleic acid activity measured on the second day is now $f(\frac{1}{2}) (\Phi \frac{1}{2})$. The share of the active deoxyribonucleic acid formed in the course of the second day in the total activity of deoxyribonucleic acid of the red corpuscles is somewhat different from the corresponding value during the first day and amounts to $f(\frac{1}{2}) (\Phi \frac{1}{2})$.

In a similar way the contribution of the active deoxyribonucleic acid formed during the third day to the total deoxyribonucleic acid activity can be calculated and also the share of the deoxyribonucleic acid formed in the course of the first and second day in the total activity measured on the third day of the experiment can be determined. Each consecutive day we find a new (Φ) value not represented previously.

As the deoxyribonucleic acid molecules present in the nucleated red corpuscles were found to be entirely stable, not showing any perceptible turnover, the above considerations permit one to determine what percentage, for example, of the labelled red corpuscles formed on the first

day is still present in the circulation at any later date. The percentage share of the erythrocytes formed during the first day of the experiment in the total red corpuscle content of the circulation at different dates is shown in Fig. 6.

One may be tempted to explain the results obtained by assuming a successive loss of the deoxyribonucleic acid content of red corpuscles during their life-cycle. However, an investigation of the deoxyribonucleic acid content of individual erythrocytes by RIS and MIRSKY⁽⁴⁷⁾ has shown that this value for each red corpuscle is constant within 10%.

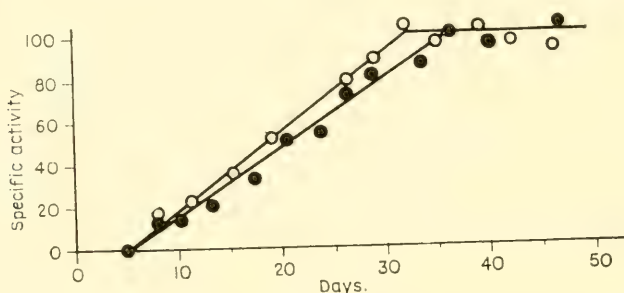


FIG. 7. — Life-cycle of the red corpuscles of two hens. Abscissae: days after start of experiment; ordinates: specific activity of deoxyribonucleic acid phosphorus extracted from the corpuscles secured at different dates (reproduced, with permission, from *Nature* **156**, 534 (1945)).

As to a possible objection that not the inorganic but an organic P fraction of the bone marrow is the pertinent precursor of deoxyribonucleic acid, OTTESEN has shown that the existence of a deoxyribonucleic acid precursor of considerably lower turnover rate than that of the inorganic P of the marrow is incompatible with the results demonstrated in Fig. 6. A precursor of more rapid turnover rate than that of the marrow inorganic P would clearly not influence the results obtained.

A method very similar to that described above had been used by SHEMIN and RITTENBERG⁽⁴⁹⁾ in the determination of the life-cycle of human red corpuscles. They administered glycine containing ¹⁵N to human subjects and followed the change with time of the ¹⁵N content of haemin isolated from the erythrocytes. They found this content to decline rapidly after the lapse of 109–127 days. Ottesen based his considerations on the ratio of the ³²P content of deoxyribonucleic acid P and its precursor (inorganic P), but SHEMIN and RITTENBERG could not

⁽⁴⁷⁾ *J. Gen. Physiol.* **33**, 125 (1939).

⁽⁴⁸⁾ *J. Biol. Chem.* **166**, 627 (1946).

follow such a procedure since the ^{15}N content of the precursor of the haemin nitrogen at different times of the experiment is not known.

SHEMIN *et al.*⁽⁴⁹⁾ applied their ^{15}N technique also to the study of the life-span of the nucleated red blood corpuscles of the chicken. The life-cycle of such corpuscles was found to be about 28 days, in good agreement with the results obtained when using deoxyribonucleic acid ^{32}P as an indicator.

Radio-phosphorus was also applied in life-span determinations in a different way, as described above.⁽⁵⁰⁾ By injecting, twice daily, labelled

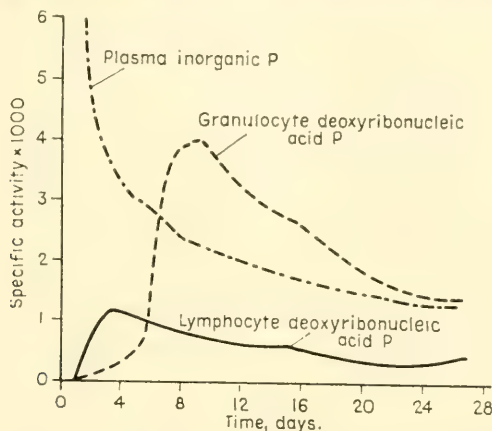


FIG. 8. — Change of the specific activity of human-plasma inorganic phosphorus and white-corpuscle nucleic phosphorus with time. (OTTESEN)

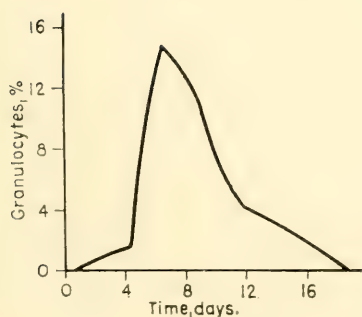


FIG. 9 — Percentage of human lymphocytes, formed during the first day of the experiment, present in the blood stream at the time indicated on the x -axis (OTTESEN).

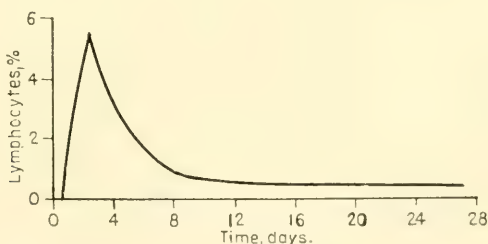


FIG. 10. — Percentage of human granulocytes, formed during the first day of the experiment, present in the blood stream at the time indicated on the x -axis (OTTESEN).

⁽⁴⁹⁾ Cold Spring Harbor Symp. Quant. Biol. **13**, 185 (1948).

⁽⁵⁰⁾ HEVESY and OTTESEN, *Nature* **156**, 534 (1945).

sodium phosphate into the hen, the specific activity of the plasma inorganic P was kept at a fairly constant level. As demonstrated by Fig. 7, after the lapse of 33 days the specific activity of deoxyribonucleic acid no longer increased, indicating that only red corpuscles formed during the experiment were now present in the circulation. As significant amounts of labelled red corpuscles were observed only 4 days after the start of these experiment, the life-span of the erythrocytes works out again to be 29 days.

(b) Life-time of Human White Corpuscles

The same considerations as described above were applied by Ottesen in the determination of the life-time of human lymphocytes and granulocytes. His results are illustrated by Figs. 8, 9, and 10. In these experiments, not the specific activity of inorganic P of the blood plasma but the corresponding value of the urine inorganic P was determined, as well as the specific activity of deoxyribonucleic acid P of lymphocytes and granulocytes. As with nucleated erythrocytes, the deoxyribonucleic acid molecules present in the white corpuscles were found to remain unchanged during the life-time of the corpuscles, all turnover being absent. Correspondingly, the ratio of the labelled deoxyribonucleic acid content of the white corpuscles formed during the first day to that of the totality of circulating white corpuscles is identical with the ratio of the number of white corpuscles formed within the first day to the total number of white corpuscles present in the circulation. It requires a few days before the white corpuscles are formed and released into the circulation. Owing to this, the labelled granulocyte content of the circulation first increases and reaches a maximum after the lapse of 6 days. About 14% of the granulocytes then present are such as were formed during the first day. Their number decreases, however, fairly rapidly with time. After a further 4 days their proportion is reduced from 14 to 7%.

A closer investigation of the number of lymphocytes formed during the first day, and still present in the circulation, revealed that an appreciable part of lymphocytes present is formed many months before the start of the experiment; thus lymphocytes contain an appreciable proportion of cells of long life. This conclusion is based on the figures obtained for the ratio of the specific activities of plasma (urine) inorganic P and the corresponding value of deoxyribonucleic acid P extracted from lymphocytes. This ratio should be equal to or less than unity for all lymphocytes formed during the experiment. A ratio appreciably higher than unity was, however, found even after the lapse of more than a month.

DETERMINATION OF THE AMOUNT OF CIRCULATING RED CORPUSCLES

Although the determination of the life-cycle of the red corpuscles necessitates a type of labelling which remains in the corpuscles throughout their life, the measurement of the amount of red corpuscles circulating in the body can be carried out in a few minutes. Hence it suffices to fix the radioactive label to the corpuscles for a comparatively short time. This procedure can be carried out *in vitro*⁽⁵¹⁾. We secure a blood sample of a human subject, add a few microcuries of labelled sodium phosphate of negligible weight, and shake the material for 1 hour at body temperature. Let us denote the number of red corpuscles injected into the circulation by A , and the ratio of red corpuscles in 1 gm of the injected blood to those in 1 gm of blood secured from the circulation after the injection by B ; then the total amount of red corpuscles present in the circulation (x) is given by $x = AB$.

If to 100 ml. of a blood sample kept at body temperature we add labelled sodium phosphate of negligible weight, about one-third of the ^{32}P atoms added are found in the red corpuscles after 1 hour. It follows that in 1 hour—it being assumed that the inorganic P content of the plasma is 4 mgm % and the weight of the plasma constitutes 55% of that of the blood—about 0.7 mgm of inorganic P moves from the plasma into the corpuscles, and *vice versa*. In the course of this interchange some of the ^{32}P added to the plasma penetrates into the red corpuscles and is replaced by ^{31}P atoms moving in the opposite direction.

The red corpuscles contain appreciable amounts of labile organic phosphorus compounds. In glycolytic and other enzymic processes taking place in the erythrocytes these compounds are degraded and resynthesized at a remarkable rate. Shortly after their intrusion as inorganic phosphate most of the ^{32}P atoms participate in the resynthesis of labile organic phosphorus compounds and are incorporated with them⁽⁵²⁾. The presence of a comparatively large amount of labile organic phosphorus molecules makes it possible to fix ^{32}P in red corpuscles during an interval which amply suffices to carry out a determination of the circulating erythrocyte volume.

If we suspend the labelled corpuscles obtained in inactive plasma, or inject them into an inactive circulation, the interchange of inorganic P between the labelled red corpuscles and the inactive plasma continues, involving respectively 0.7 mgm of inorganic plasma and corpuscle P in 100 ml. of blood per hour. While, however, during the activation process,

⁽⁵¹⁾ HEVESY and ZERAHN, *Acta Physiol. Scand.* **4**, 376 (1942); BOHR, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **13**, Nr. 1 (1950).

⁽⁵²⁾ ATEN and HEVESY, *Nature*, Lond. **142**, 871 (1938).

together with 0.7 mgm of inorganic phosphorus, ^{32}P of negligible weight and having an activity of $1\ \mu\text{c}$. moves into the corpuscles, after injection of the labelled corpuscles into an inactive circulation the migration of 0.7 mgm of inorganic P from the red corpuscles into the plasma will be followed by that of about $0.1\ \mu\text{c}$. only (cf. Figs. 11 and 12). This is because most of the ^{32}P (and ^{31}P) after having penetrated into the red corpuscles finds a temporary abode in the organic phosphorus compounds

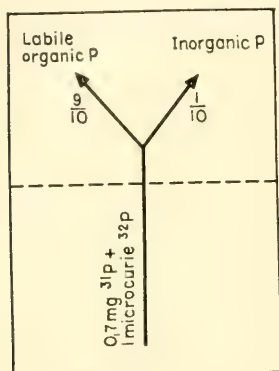


FIG. 11. — Distribution of inorganic phosphate intruded into the red corpuscles between organic and inorganic fractions

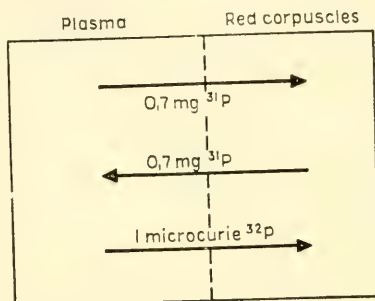
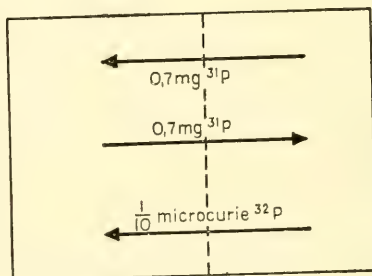


FIG. 12. — Interchange of ^{31}P and ^{32}P between plasma and red corpuscles



present in the erythrocytes. About $1/10$ represents the ratio of the inorganic P content of the plasma and labile P content of the red corpuscles. This ratio, and correspondingly the time during which ^{32}P is kept in the erythrocytes, may vary for the blood of different subjects. The permeability of the red corpuscle membrane, which is also of importance for the temporary conservation of ^{32}P in the corpuscles, may vary as well. The loss of the ^{32}P content of the corpuscles in the course of 20 minutes is, however, in no case larger than 3%, and in most cases even appreciably less. When not labelled corpuscles but labelled blood is injected into the circulation, loss of ^{32}P by the corpuscles during the experiment can furthermore be compensated to an appreciable extent by uptake of ^{32}P from labelled plasma present in the circulation.

(53) *Ark. Kemi* **20**, A Nr. 17 (1945); cf. REEVE and VEALL, *J. Physiol.* **103**, 12 (1949).

NYLIN⁽⁵³⁾ made a very extended application of the method described. He determined, besides the total circulating erythrocyte volume, that of single organs such as lungs and legs. A blood sample of a human subject was secured and labelled with ^{32}P as described above. Before injection of an aliquot of the labelled red corpuscles into the subject, the vessels were clamped. The red corpuscles circulating in the legs were thus prevented from participating in the "dilution" of the injected labelled erythrocytes, which takes place after injection of labelled red

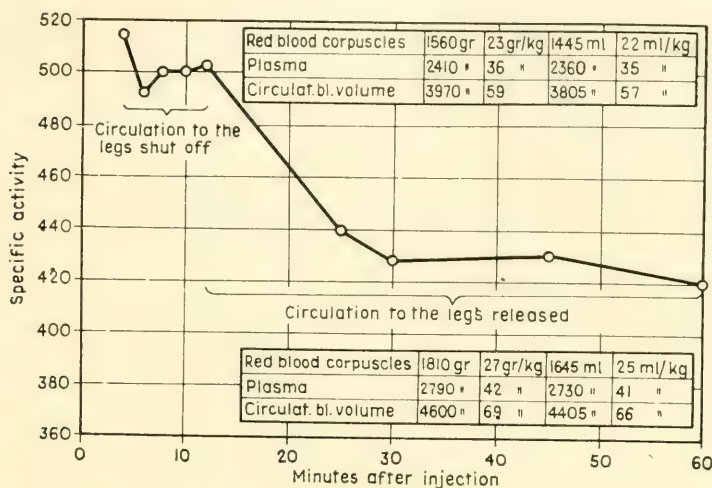


FIG. 13. — Determination of the circulating corpuscle (blood) volume of the legs (Nylin) (reproduced, with permission, from *Ark. Kemi* 20, A No. 17 (1945).

corpuscles into the non-radioactive circulation and the extent of which indicates the amount of circulating erythrocytes. As seen in Fig. 13, 10 minutes after injection of labelled corpuscles, the amount of circulating erythrocytes, excluding that of the legs, is found from the "dilution" figures to be 1560 gm. After removal of the clamps, there was a marked decrease in the activity of a 1 gm sample of corpuscle (denoted in Fig. 13 as specific activity). Owing to the participation of the red corpuscles of the leg in the "dilution" process, the specific activity of the corpuscles decreases from 550 to 428, indicating that the red corpuscle volume of the body, which now includes that of the legs as well, amounts to 1810 gm. From the above figures the weight of the erythrocytes circulating in the legs is determined to be 250 gm.

The labelling of a red corpuscle sample *in vitro* is made possible by the presence in the red corpuscles of an enzymic mechanism which is instrumental in alternately degrading and building up the comparatively large amounts of labile organic phosphorus compounds in the erythros

cytes, and also by the low inorganic phosphate content of the corpuscles, and finally by the fairly slow rate of penetration of phosphate from the plasma into the corpuscles, and *vice versa*.

Red corpuscles can also be labelled by introducing radio-iron, ^{55}Fe or ^{59}Fe . Iron-labelled red corpuscles keep their label throughout their life. As, however, the labelling of erythrocytes with radio-iron can only be carried out *in vivo* it necessitates having blood donors to whom iron of appreciable radioactivity must be administered. Iron-labelled red corpuscles are not applied in the determination of the erythrocyte content of the human circulation. Such corpuscles proved to be useful, *inter al.*, in the study of the post-transfusion survival of erythrocytes preserved during several weeks.

APPLICATION OF ^{14}C IN THE STUDY OF METABOLIC DEPRESSORS AND ACCELERATORS

Although the rapid change in the sensitivity of the indicator during the experiment may be disturbing, as in the study of the mineral constituents of the skeleton (see p. 982), it is most advantageous when we are interested in restricting the formation of labelled components to a short time interval. This is the case, as we saw, in the study of the life-

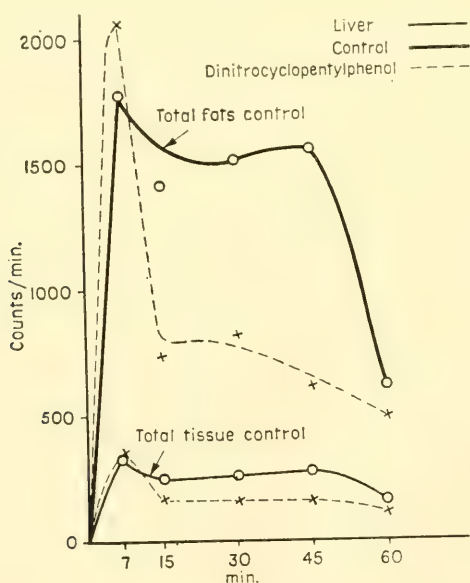


FIG. 14. — Effect of dinitrocyclopentylphenol on the incorporation of ^{14}C into total fats and total tissue of mice liver. Groups of animals killed at different times after injection of $\text{CH}_3 \cdot ^{14}\text{CO}_2\text{Na}$ (reproduced, with permission, from *Arch. internat. Pharm. Therap.*, **36**, 33 (1951).

cycle of blood corpuscles. The rapid decrease with time in the specific activity of the precursor proves also to be useful in the study of the change of the metabolic rate produced by metabolic depressors or accelerators.

Numerous carbon compounds present in the organism are metabolized at a spectacular rate. Though, as shown by BLOCH and RITTENBERG⁽⁵⁴⁾,

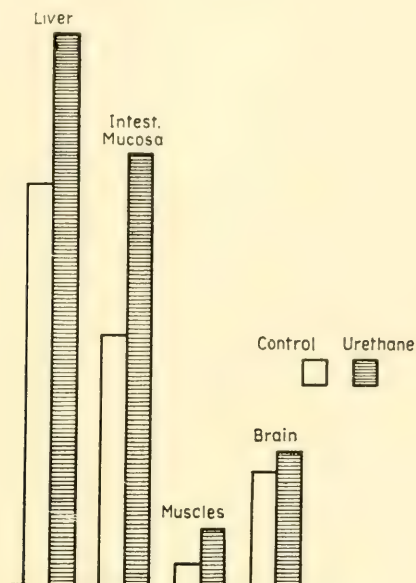


FIG. 15. — Effect of urethane (20 mgm) on the incorporation of ^{14}C into tissue phosphatides. Mice killed 100 min, after injection of $\text{CH}_3 \cdot ^{14}\text{CO}_2\text{Na}$.

a 100 gm rat by catabolizing fatty acids produces 1 gm of acetate and other tissue constituents per day, the body acetate level of the organism remains very low, owing to the rapid utilization of the acetate produced. This compound is a precursor of acetoacetic acid, cholesterol, fatty acids, glycogen, the dicarboxylic amino-acids, proto-porphyrin, uric acid, and the acetyl group formed in many acetylation reactions, with some of which the ^{14}C atoms of acetate are incorporated at a remarkable rate. If we inject labelled acetate into the mouse, the injected radioactive acetate is diluted by non-radioactive endogenous acetate. This dilution rapidly increases owing to the fact that, while the radioactive acetate injected only at the start of the experiment is rapidly metabolized, the endogenous non-radioactive acetate, though rapidly metabolized as well, is newly formed throughout the experiment. The decrease in the

⁽⁵⁴⁾ *J. Biol. Chem.* **159**, 45 (1945).

specific activity of the body acetate is necessarily reflected in the specific activity of those products with which acetate carbon is incorporated. As resorption, distribution, and conversion of acetate take some time, the ^{14}C content of a rapidly metabolizing fatty acid fraction found to be present in the liver of the mouse after intraperitoneal injection of labelled acetate increases during the first minutes; soon, however, a very rapid decline in the activity figure of the fatty acids is observed⁽⁵⁵⁾. The strongly active fatty acid¹⁸ molecules formed in the initial phase of the

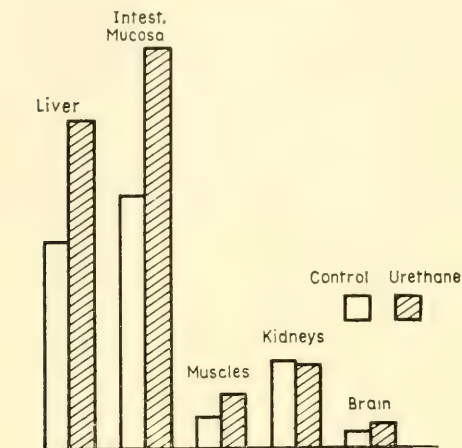


FIG. 16. — Effect of urethane on the incorporation of ^{14}C into total fat. Mice killed 110 mins. after injection of $\text{CH}_3 \cdot \text{CO}_2\text{Na}$.

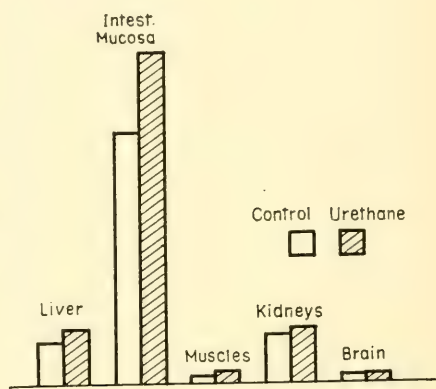


FIG. 17. — Effect of urethane on the incorporation of ^{14}C into tissue proteins. Mice killed 110 mins. after injection of $\text{CH}_3 \cdot ^{14}\text{CO}_2\text{Na}$.

experiment are soon metabolized and replaced by molecules formed from less active precursors; correspondingly, a rapid decrease in the fatty acid activity takes place.

Dinitro-compounds, e.g., dinitro*cyclopentyl*phenol, when given in proper doses, are metabolic accelerators. Consequently, they enhance the incorporation of acetate ^{14}C into liver fats of the mouse, as seen in Fig. 14.⁽⁵⁵⁾ Soon, however, the accelerated metabolism leads to an accelerated replacement of the labelled fatty acid molecules (in which most of the ^{14}C of the total fats is to be found) formed in the early phase of the experiment by molecules formed from less active precursors. The descending part of the curve correspondingly takes a steeper course in the case of the dinitro*cyclopentyl*phenyl-treated animals than in that of the

⁽⁵⁵⁾ HEVESY, RUYSSSEN and BEECKMANS, *Experientia* (1951), BEECKMANS and DE ELLIOTT, *Nature* **167**, 200 (1951).

⁽⁵⁶⁾ BEECKMAN, CAISER and HEVESY, *Arch. Int. Pharmacodyn.* **86**, 33 (1951).

controls. Urethane, which is a metabolic depressor, acts in an opposite way⁵⁷. In experiments taking 100 minutes, thus in the descending region of the curve (Fig. 14), the phosphatide fractions or the total fat (Fig. 16) extracted from various organs of urethane-injected mice take up more ^{14}C than those of the controls. A similar, but less pronounced effect is found (Fig. 17) when comparing the incorporation of ^{14}C into the proteins of organs of urethane-injected mice with the incorporation into corresponding fractions of controls.

In a similar way, acetate labelled in the carboxyl group with ^{14}C was applied in the study of the biochemical effects of ionizing radiation⁽⁵⁸⁾. These investigations revealed an interference, not only, as mentioned previously with the rate of incorporation of ^{14}C into purines of nucleic acids, but also of other tissue constituents. In the *non-fasting* mouse, for example, irradiation produced a similar effect on the incorporation of ^{14}C into fats and proteins of the brain to that of administration of urethane.

While the disentanglement of the numerous, often competing, metabolic steps involving ^{14}C incorporation and the determination of the extent to which this incorporation is influenced by metabolic depressors and accelerators is a very difficult task, the method outlined above may prove to be a promising approach to the study of metabolic interferences.

The application of radioactive indicators in biochemistry covers a large field and I had to restrict myself to the discussion of a few examples only.

Possible interference of ionizing radiation with the normal biochemical pattern is often considered a disadvantage of the method of radioactive indicators. Such an interference can, however, be avoided by using radioactive tracers of restricted activity. A much more dangerous source of error in the application of radioactive indicators is the non-identity of the chemical properties of isotopes. Tritium, for example, having an atomic mass three times as high as hydrogen, differs from hydrogen to a non-negligible extent in many of its chemical properties. We are witnessing the establishment of a new branch of chemistry which in the course of time may become a very important implement for classical chemistry, namely, that of the rare isotopic constituents. In the long run the claim for accuracy in the biochemical application of isotopic indicators is bound to increase and it may become necessary to make use of the advances in this new branche of chemistry.

The application of isotopic indicators in biochemistry opened new lines of approach, not only to the solution of known problems, but also

(57) HEVESY, *Nature* **164**, 1007 (1949); HEVESY, RUYSSSEN and BEECKMANS, *Experientia* (1951).

(58) HEVESY, *Nature* **163**, 869 (1949).

by directing our attention to trains of thoughts not considered previously. Some of these ideas I have tried to outline in this lecture. It also induced the experimental chemist to take an interest in the history of the atoms and molecules with which he is dealing.

The chemist is not a historian, he is not interested in the problem whether the carbon atoms of the benzene he is experimenting with were formerly stored in Welsh coal deposits, in carbon dioxide of a volcanic outburst, in the carbonate of crustacea shells, or in a mammalian skeleton. In contrast to the classical chemist, the indicator chemist is to some extent a historian, highly interested in the past of atoms, molecules, and molecular aggregates. He has a great concern in the distinction of how far molecules present in the tissue are "old" or "new". He wishes to know when the potassium atoms present in the tissue cells left the circulation, when the nucleic acid molecules present in the nuclei of thymus cells were formed. He is interested in questions like the former abode or abodes of the carbon atoms of glycogen, the origin of faeces constituents, whether they originate from undigested food or are due to endogenous secretion. He may desire to know which calcium, phosphorus, nitrogen, or sulphur atoms of the plant originate from the soil and which from the added fertilizer, possibly even which from the fertilizing pollen.

Many of the problems attacked by the tracer chemist are such as had been solved previously. The application of isotopic indicators often led to a remarkable simplification of earlier methods. This is the case when measuring circulation rates, water contents, blood volumes, etc. On the other hand, the application of isotopic indicators opened the only way to determine the rate, place, and sequence of formation of many molecular constituents of the living organism. The very existence of such methods was instrumental in opening new trains of thought, in demonstrating the dynamicity of metabolic processes, in confirming HOPKINS's statement that "life is a dynamic equilibrium in a polyphasic system," in concentrating our interest on the problem of the velocity of the fundamental biological processes.

Lord RUTHERFORD, who—as NIELS BOHR, the preceding Faraday lecturer, so appropriately stated—contributed more than anyone else to the most remarkable development which followed BECQUEREL's discovery, remarked, when delivering his Faraday Lecture in 1936: "We can now look back with some sense of perspective and recognize the extraordinary importance of the discovery of radioactivity and the profound influence on a knowledge of atoms and the relation of the elements which has followed from a detailed study of the radioactive bodies." It was this detailed study and the following most remarkable development which made possible the application of radioactive substances as indicators.

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Lecture delivered in 1956 at the international meeting of nuclear medicine in Turin.

100. HISTORICAL PROGRESS OF THE ISOTOPIC METHODOLOGY AND ITS INFLUENCES ON THE BIOLOGICAL SCIENCES

G. HEVESY

By adding a radioactive isotope to atoms or molecules we can label these and follow their path. That the labelling device was bound to find a very extended field of application was clear already in 1913 when it was first applied. With increasing time and increasing availability of radioactive isotopes the field of application of radioactive indicators immensely widened and is to be expected to expand further in the years to come.

For the first decade, radioactive indicators found application in the realm of inorganic and physical chemistry only. But the introduction of some of the leading principles applied in the later biological studies goes back to these early times. The method of isotope dilution, for example, which finds such an extended application in biochemical, physiological and clinical studies goes back to these early days. The first application of radioactive indicators in plant and animal physiology was carried out in 1923. Both the uptake of with ThB or RaD labelled lead by bean seedlings and its release by the plant when placing it in a culture solution containing non-radioactive lead was studied. This was soon followed by the study of the uptake of labelled atoms by the animal organism.

In the investigations carried out up to 1933 only the isotopes of lead, bismuth, thallium, radium, thorium and actinium were applied as tracers, thus isotopes of a very restricted number of elements and none of them a main constituent of the living organism. But lead isotopes as tracers found even in the recent times new useful applications in physiological and clinical studies. The lead isotope ThB proved to be a useful label of red corpuscles and RaD found application in the study of the conservation of lead by the all-through-labelled skeleton of mice obtained by administering to the mother RaD and also to the offsprings until adult age was reached.

In 1932 and 1933 most fundamental discoveries were made which were bound to deeply impress the very few workers who at that date were interested in the application of isotopic tracers. These discoveries were

that of deuterium by UREY and the production of artificial radioactivity by the JOLIOT-CURIE'S.

Due to the great kindness of Professor UREY we obtained, shortly after his discovery of deuterium, diluted (0.5 mol %) heavy water and could embark on the investigation: if and at what speed the water molecules of the goldfish interchange with the water molecules of their surroundings, and on the determination of the mean-life time of water molecules of the human body and of its water content. In the last mentioned determination⁽²⁰⁾ the method of isotope dilution being used which was first applied when determining the minute amounts of lead present in rock samples. If we administer 10 ml of heavy water, after the lapse of some hours when the administered heavy water gets into exchange equilibrium with the body water we find 1 ml of the latter to contain, beside the minute amounts present in all water, 1/5000 part of the administered heavy water, and the total water volume of the body works out to be 50 litres.

A year later concentrated heavy water became available and the above mentioned studies were soon followed by others of greatest importance carried out by SCHOENHEIMER and RITTENBERG. The first showed that an appreciable part of the deuterium labelled higher fatty acids administered to the mouse can be located in the fat deposits even if the animal is losing weight. They demonstrated with these studies the dynamic nature of fat deposits. These are constantly renewed. Their investigations much enlarged our knowledge of fat metabolism. They embarked then on the study of protein metabolism, applying ¹⁵N as a tracer which was determined, as was the deuterium content of the compounds investigated, with the mass spectrograph. The results obtained in the last mentioned studies, demonstrating the dynamic nature of protein metabolism, proved to be of fundamental importance as well. This important subject will be discussed by Professor MACFARLAINE who made numerous contributions of great interest to this field. Though we know today that the dynamic nature of protein metabolism is confined to the metabolic proteins, thus to a fraction of the proteins present in the organism only, this fact in no way reduces the great importance of SCHOENHEIMER and RITTENBERG's early results.

As already mentioned the production of artificial radioactive substances was discovered about the same time as deuterium. FRÉDÉRIC JOLIOT's and IRÈNE CURIE's fundamental discovery was followed at once by a study of FERMI and his colleagues who produced a very great number of radioactive isotopes under the action of neutrons emitted by mixtures of radon and beryllium. In the first application of an artificial radioactive isotope, that of phosphorus, the same method was used in Copenhagen. Glass tubes containing radonberyllium mixtures were immersed in 10 litres of carbondisulfide which absorbed a large fraction

of the neutrons emitted transforming some of the ^{32}S atoms of the carbondisulfide into radioactive P atoms. By extracting the carbondisulfide with diluted acid or with water a solution of ^{32}P was obtained and first applied in 1935 in the investigation if and to what extent the phosphorus atoms of the mineral constituents of the skeleton are replaced. Within 24 days 30% of these were found to be renewed. As about 300 mgm bone phosphorus were involved in the renewal process and the total phosphorus content of the blood of the rat amounts to not more than 10 mgm, most of the phosphorus atoms which entered the mineral constituents of the skeleton must have been previously located in the organs. These experiments⁽¹⁾, in which artificial radioactive tracers found their first application, thus brought out the dynamic state of the phosphorus compounds of the organs as did simultaneously SCHOENHEIMER and RITTENBERG's investigations, applying a stable isotopic tracer, the incessant rejuvenation of fatty acids and other molecular body constituents. It was soon made clear that isotopic methods offer the only possibility of studying the organism as a whole under practically equilibrium conditions.

The ^{32}P available in the earliest investigations with artificially produced radioactive indicators, though of very modest activity (less than $1\ \mu\text{C}$) was found to be useful in numerous investigations. It sufficed even to carry out the first clinical red corpuscle volume determination. For numerous other investigations these minute activities did, however, not suffice, for example to follow, after injecting radiophosphorus to the goat, the incorporation of ^{32}P into the various phosphorus compounds of the milk. These and many other investigations became only possible after the availability of cyclotron-produced radiophosphorus. While the availability of pile-produced radioactive isotopes alone made possible the exceedingly extended application of radioactive tracers which we witness today, the construction of the cyclotron was one of the greatest events in the history of applications of radioactive indicators. In 1939 already, only four years after the first application of ^{32}P in physiological studies, the first paper of JOHN LAWRENCE and his collaborators appeared⁽²⁾ in which cyclotron-produced radiophosphorus was applied in clinical studies of leukemia followed by numerous communications on various clinical topics in which radioactive isotopes were applied by them and others as indicators. We shall discuss later some important work carried out by the Berkeley group. In 1939 furthermore a paper was published in Copenhagen on the resorption of phosphate from the human intestine. By comparing the specific activity of the urine and faeces P, it was possible to distinguish between the endogenous and exogenous phosphate present in the faeces⁽³⁾.

It was cyclotron-produced iodine which HAMILTON⁽⁴⁾ applied in his pioneer studies of radioiodine uptake by the thyroid. Radioactive

sodium, potassium, calcium, strontium, carbon 11, sulfur and so on became available and found application in permeability and other studies. Skeleton metabolism could now be studied even applying calcium and strontium. Both iron 55 and 59 were prepared. ERNEST LAWRENCE was most generous towards the few workers then applying radioactive tracers and supplied them with radiophosphorus and a few other radioelements. When we learned that he had succeeded in preparing radioiron we were very anxious to obtain some. He told us, however, that he had promised WHIPPLE to put all the restricted amounts of radioiron available at his disposal. This was a very wise decision in view of the important work which the WHIPPLE—HAHN group carried out in following resorption and incorporation of radioiron and therewith opening one of the most beautiful chapters of the physiological and clinical application of radioactive indicators.

It looked for a while as if, when looking for suitable tracers for the chief constituents of the living organism, for hydrogen, carbon, nitrogen and oxygen, we would have to make use of stable rare isotopes of ^2H , ^{13}C , ^{15}N and ^{18}O which are also useful but very much more elaborate and less sensitive to determine than the radioactive ones. Soon, however, making use of the cyclotron, tritium (ALVAREZ and CORNOG, 1939) and the long living carbon 14 (RUBEN and KAMEN, 1940) were discovered. To make them available in a sufficient amount the immense neutron flux was necessary which alone the pile can supply. The fission products, the most copious source of radioactive substances, does not contain the above mentioned or other light elements.

Isotopic indicators found application in all branches of biology in the elucidation of a great variety of problems and were opening entirely new approaches. One of these of special importance was the splitting of dynamic equilibria so frequent in the living organism. The glucose content of the circulation, for example, is the result of glucose taken up or produced by the organism and that metabolised. An abnormally high glucose level met in diabetes may be due to a depressed utilization or to enhanced formation of this compound. The application of isotopic indicators permits to distinguish between the high glucose level due to impaired metabolism and that due to increased formation. Some of these investigations were carried out by studying glucose metabolism in tissue slices. HASTINGS and associates⁽⁵⁾ incubated in a set of experiments liver or muscle (diaphragm) slices of the rat with ^{14}C labelled glucose in the presence of non-labelled pyruvic acid. In another set of experiments the tissue slices were incubated with non-labelled glucose and with ^{14}C labelled pyruvic acid. The first set of experiments aimed at the determination of glucose uptake by the liver or the muscle and its transformation into glycogen, fatty acids and other components. The last mentioned to obtain information about the amount of glucose built up

in the organism, pyruvic acid being an intermediary of glucose formation. Glucose metabolism in tissue slices from normal and from insulin-injected diabetic rats, killed from 15 minutes to 48 hours after the first insulin injection, was compared. Metabolism of labelled glucose involves the formation of labelled glucose-6-P. As seen in Fig. 1 minute amounts of labelled glucose-6-P molecules are formed only in the liver of the diabetic rat prior to insulin injection. After the first injection it takes 10 hr until glucose-6-P is formed at a normal rate; later an accelerated glucose metabolism leading to hypoglykemia is observed.

Labelled glucose formation from labelled pyruvate is, as seen in Fig. 2, markedly enhanced in the diabetic liver and it takes about 30 hours after the first injection of insulin before it is depressed to its normal

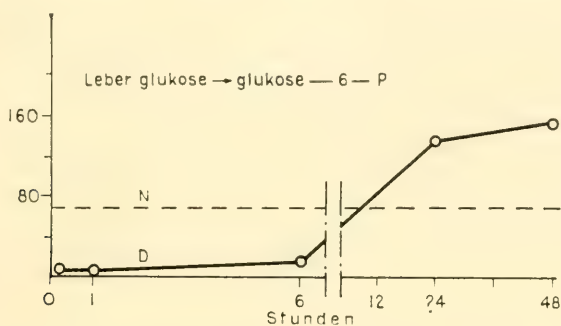


FIG. 1. — Effect of administration of insulin to the rat on the rate of conversion of glucose- ^{14}C into labelled glucose-6-phosphate. — (N: normal; D: diabetic)

value. Later, glucose formation is brought below its normal level leading to hypoglykemia, as does, as mentioned above, the abnormally increased utilization of glucose. The increased glucose formation in the diabetic animal was also shown in experiments in which the conversion of the ^{14}C of palmitic acid into that of glucose was made. The rate of formation of labelled glucose was four times higher in the diabetic rat.

The formation of glucose in the organism was further investigated by STETTEN⁽⁶⁾ in experiments in which the labelled glucose level of the organism was kept constant during the experiment by continuous intravenous injection. The non-labelled glucose, formed in the organism, dilutes the activity of the urinary glucose and this dilution is a measure of the glucose formation.

Denoting with R the rate of injection in mgm of glucose per hour per rat, and A and a denoting the specific activities of injected and excreted glucose respectively, the amount of glucose formed from non-isotopic precursors, thus not through resynthesized split-products of the injected labelled glucose, $r = R (A/a - 1)$.

In the normal 100 gm rat 15 mgm glucose were found to build up per hour, in the diabetic rat 21 mgm. The formation of glucose from sources not derived from the infused glucose was found to be enhanced up to 7-fold after administration of cortisone.

Clinical investigations on glucose turnover applying ^{14}C labelled glucose were carried out as well⁽⁷⁾. From the change of the specific activity of the exhaled CO_2 and of the plasma glucose with time it was calculated that 31 per cent of the CO_2 expired derived from oxydation of glucose in normals. In diabetics not showing ketosis this percentage was somewhat depressed to 27 but in those showing ketosis, to the markedly depressed value of 19. A normal human subject weighing 70 kgm was found to

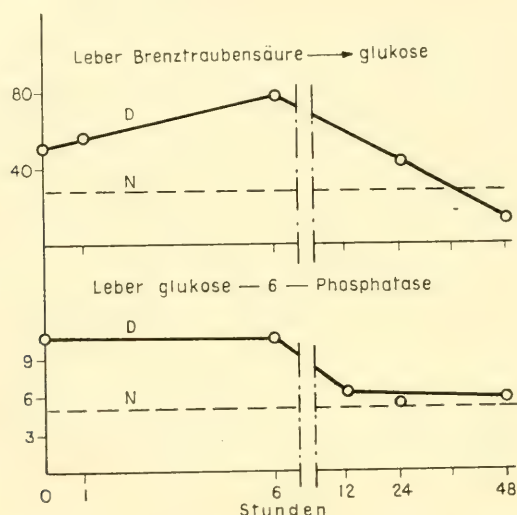


FIG. 2. — Effect of administration of insulin to the rat on the rate of conversion of pyruvate- ^{14}C into labelled glucose and on the level of glucose- phosphate activity measured as milligram of phosphorus released from glucose-6phosphate per gram of liver per 30 minutes.

burn 3.8 gm glucose per hour, some of the “stable” diabetes patients almost the same, the labile ones 2.2 gm only.

In these experiments 90 μC of glucose extracted from tobacco plants which were grown in a $^{14}\text{CO}_2$ containing atmosphere were injected into the circulation. All carbon atoms of such glucose are labelled. Such an amount of ^{14}C exposes the organism to a radiation dose of 0.17 Mrep in the course of the first hour, the dose soon decreasing due to excretion of ^{14}C .

REPLACEMENT RATE OF THE IRON ATOMS OF THE PLASMA

Splitting the dynamic equilibrium, which the plasma iron content represents into its components, illustrates the wealth of biological and clinical information which can be obtained by applying a radioactive tracer, and by this way only.

As already mentioned the first application of ^{59}Fe was that of HAHN-WHIPPLE and their associates. They compared among others the incorporation of labelled iron into the erythrocytes of non-bled and bled dogs and found very much more ^{59}Fe in the latter than in the former. Since most of the absorbed iron is in time incorporated into the erythrocytes the activity of the latter is a measure of the iron absorbed from the intestinal tract. The determination of the extent of iron absorption, formerly a very tedious process, is immensely facilitated by following the incorporation of administered labelled iron into the red corpuscles. By using this method among others CHODOS and assoc.⁽⁸⁾ demonstrated recently that the quantity of inorganic iron administered to a fasting subject concomitantly with ascorbic acid is 2 to 100 times larger than that of food iron. In these experiments labelled eggs and vegetables were fed which contained comparable quantities of iron with those of the inorganic compounds (FeSO_4 or FeCl_2).

An important advance was due to the study of FLEXNER and assoc.⁽⁹⁾ who injected labelled FeCl_3 into the circulation of the guinea pig and observed that a fraction of ^{59}Fe left the plasma exceedingly rapidly, while an other fraction left at a very appreciably slower rate. The last mentioned fraction, which could be found from HOLMBERG and LAURELL's and SCHADE and CAROLINE's results, was ^{59}Fe bound to the β_1 -globulin fraction of the plasma. Only a fraction of the latter is combined with iron under physiological conditions, thus small amounts of iron added to the plasma sample have opportunity to combine with this protein.

HUFF and his associates⁽¹⁰⁾ were the first to incubate plasma with minute amounts of labelled iron, reinject the plasma sample and follow the rate at which the ^{59}Fe leaves the circulation. The reinjected labelled plasma sample is distributed within a few minutes all over the plasma and thus the disappearance of the ^{59}Fe from the plasma indicates the disappearance rate of the circulating plasma iron. This method was widely applied in very numerous investigations. By following the rate of disappearance of the iron atoms of the plasma, the iron concentration remaining constant during the experiment, we determine the rate of intrusion of iron atoms into the plasma as well. The mgm plasma iron replaced per hour =

$$\frac{0.693 \quad (\mu\text{g Fe/ml}) \times \text{plasma volume (ml)}}{\text{time of half-disappearance of plasma } ^{59}\text{Fe (in hours)} \times 1000}$$

In normal human per kgm weight 0.35—0.65 mgm/kgm iron leaves the plasma in the course of 24 hours or in a 70 kgm subject 24—45 mgm. and is replaced to a very large extent by endogenous iron finding its way into the circulation as the uptake from the intestinal tract makes out only 1—2 mgm daily. The bulk of the plasma iron finds its way into the marrow and is utilized in haemoglobin formation. If haemopoiesis is impaired it will reflect itself in a change in the amount of iron leaving the plasma and incorporated into the red corpuseles. Interference with

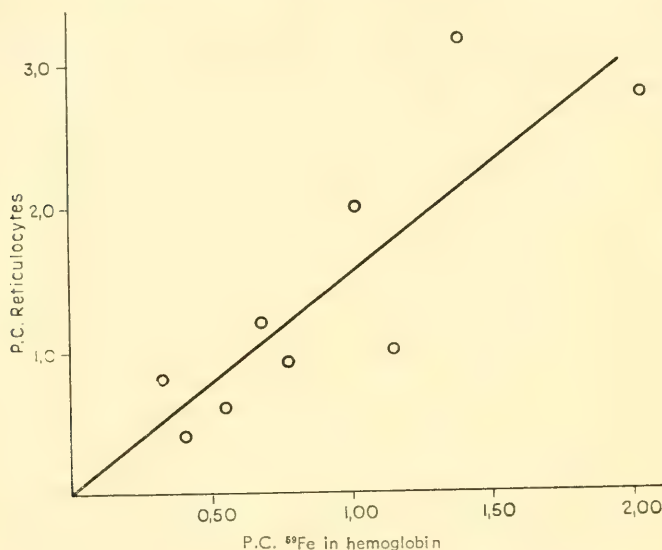


FIG. 3. — ^{59}Fe content of haemoglobin 2 hours after intravenous injection of ^{59}Fe labelled plasma plotted against the reticulocyte content of human blood.

normal haemopoiesis will reflect itself in a change in the haemoglobin content of the circulation as well. However it takes days before a noticeable change in the haemoglobin content can be ascertained, while a few hours only, or even less, after such a disturbance has set in it will reflect itself already in a change of the amount of iron leaving the circulation, which can be determined only by labelling the plasma iron and measuring the disappearance rate of ^{59}Fe from the plasma and the iron content of the latter. The ^{58}Fe incorporation into haemoglobin some hours only after labelling the plasma iron has to be interpreted very cautiously as the ^{59}Fe absorbed at that very early date in the circulating haemoglobin is not due to, or not mainly due to, incorporation of ^{59}Fe into haemoglobin in the marrow but into circulating reticulocytes and is a measure of the number of the latter as seen in Fig. 3, taken from a paper of my collaborator DAL SANTO⁽¹²⁾.

The two very instructive examples of a strongly increasing rate of exodus and replacement of iron atoms from the plasma demonstrated by Figs. 4 and 5 are both taken from papers by LAWRENCE⁽¹³⁾ and his associates. One demonstrates the strongly enhanced plasma iron turnover rate when students are moved from sea level to a height of 5000 m. The other the very marked exodus and replacement of plasma iron in polycythemia vera patients and the slowing down of this process after administration of ^{32}P which depresses the hyperplasia of the bone marrow

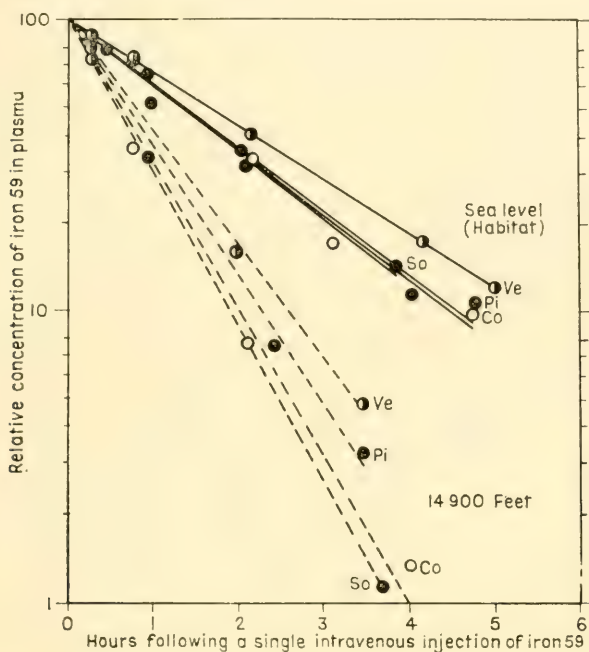


FIG. 4. — Plasma iron turnover of students at sea level and at 14,900 feet.

which is responsible for the increased red corpusele formation in these patients leading to an increased iron demand, thus a plasma iron flow at a strongly enhanced rate.

DAL SANTO⁽¹³⁾ studied in our laboratory the change in the rate of the replacement of iron atoms in the plasma of neoplastic patients. The subjects investigated were 23 patients of Dr. KOTTMAYER, the director of the gynaecological department of the Radiumhemmet in Stockholm, all suffering from cancer of the uterine cervix in the first, second and third stage of development. As seen from Fig. 6 ^{59}Fe and thus all iron atoms present leave the plasma in most cases at a markedly enhanced rate. As however the iron content of the plasma of the subjects investigated was in most cases markedly lower than in normals, the mgm iron

which reached in the time unit the bone marrow of the neoplastic subject was in many cases not larger than in normals. In Fig. 7 the plasma iron concentration as a function of plasma ^{59}Fe $T_{1/2}$ is plotted. When drawing this figure, for the sake of simplicity, the difference in the plasma volume

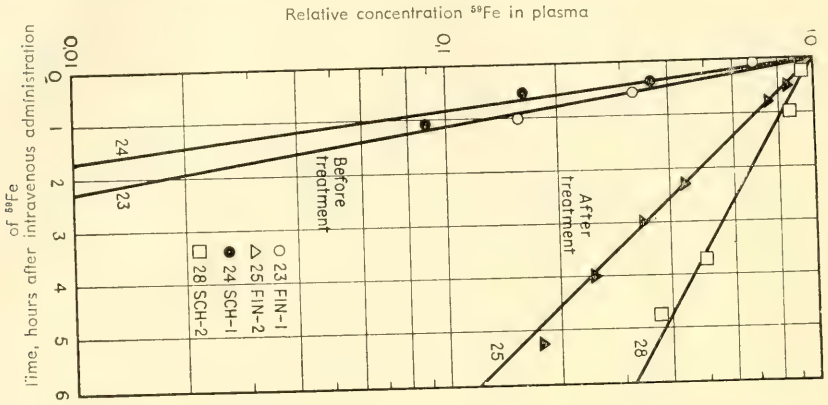


FIG. 5.

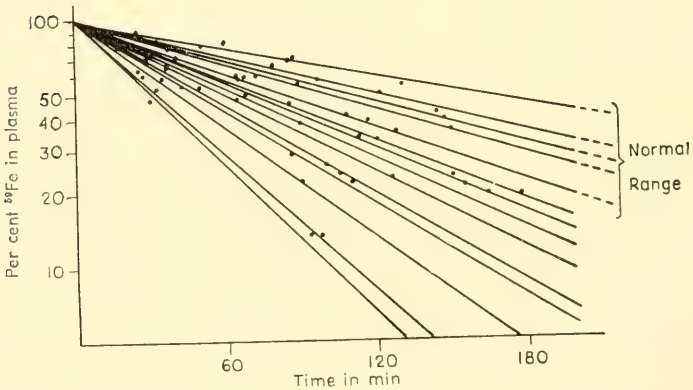


FIG. 6. — Rate of disappearance of labelled plasma iron from the circulation of patients suffering from cancer of the uterine cervix.

of the women investigated was disregarded. The mean value of the latter was 2580 ml. $M = \pm 456$. In a healthy subject we can expect at a normal iron level a normal Fe turnover and if the plasma iron content is depressed, a correspondingly increased rate of exodus of plasma iron and vice versa. Twelve of the patients show this correlation. As 7 of these were anaemic, in these cancer patients a fractional iron turnover in normal range is thus not sufficient to prevent anaemia, probably due to a shortened life-cycle of their red corpuscles which requires an additional iron turnover. In 5 other cases the fractional iron turnover rate

was higher than to be expected in view of the comparatively high plasma iron figures. This result strongly suggests the explanation that the life-cycle of the red corpuscles of these patients was shorter than normal.

That the life-span of the red corpuscles of these patients was markedly shortened could be shown by Dal Santo by labelling these with ^{51}Cr *in vitro*, reinjecting the labelled erythrocytes and following the rate of loss of their ^{51}Cr content. The label was lost more rapidly by the red corpuscles of most patients investigated than by the red corpuscles of normals, as seen in Fig. 8, indicating a shorter life-cycle of the red corpuscles of the cancer patients. A similar observation was made in other

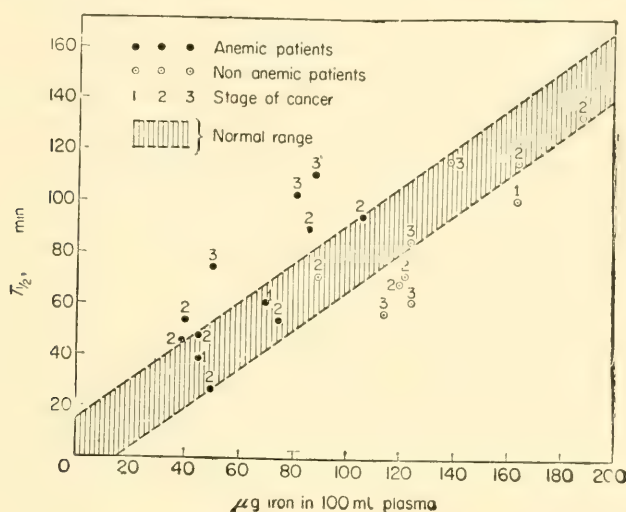


FIG. 7. $T_{1/2}$ of the extrusion rate of labelled iron atoms from the plasma plotted against the plasma iron level.

investigations as well. A shortened life-span of erythrocytes in leukemia patients was in several cases observed by Berlin and his associates¹⁴. Miller et al.¹¹ investigated patients with leukemia and malignant lymphoma and found 2/3 of these to show an increased red cell destruction. In 15 out of 19 patients in a progressive cancerous state HYMAN and HARVEY⁽¹⁶⁾ found a shortened life-cycle of the erythrocytes.

In many cases the shortened life-cycle of the red corpuscles does not lead to a corresponding anaemia, as it is partly or wholly compensated by an increased formation rate of red corpuscles. This fact is conspicuously demonstrated by the results of animal experiments. In our laboratory G. v. EHRENSTEIN injected several hundred mice with glycine- ^{14}C -2 and compared the effect of inoculation of ascites cancer cells 5 days later on the life-span of red corpuscles which were formed, thus labelled, in a non-cancerous milieu. Groups of mice were killed at

intervals and the activity of 20 mg haemin isolated from the red corpuscles compared. His results are seen in Fig. 9. The cancerous environment shortened the life-cycle of the erythrocytes of the mouse from 40

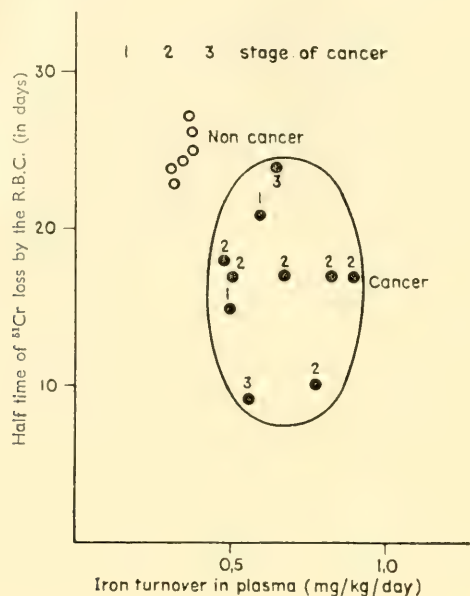


FIG. 8. — Loss of ^{51}Cr by the *in vitro* labelled and reinjected red corpuscles in the circulation of normals and of such with cervix uterine cancer.

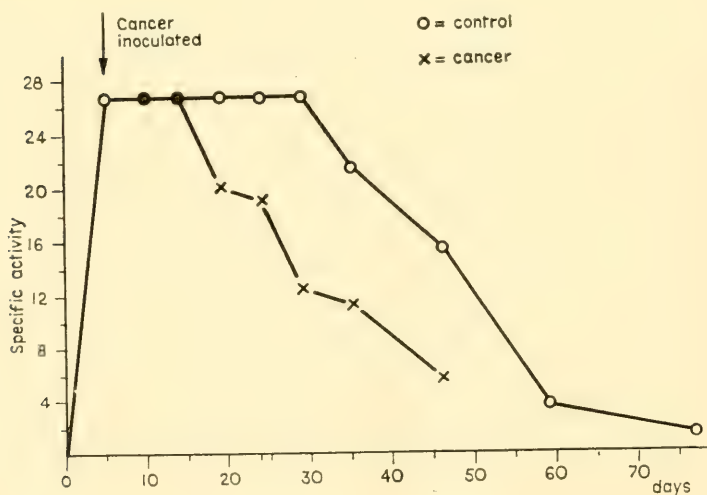


FIG. 9. — Loss of ^{14}C by the haemin of the red corpuscles of control mice and such inoculated with ascites cancer cells 5 days following injection of glycine- ^{14}C -2. The figure is showing the effect of cancer on red corpuscles formed in the non-cancerous organism.

to 20 days. In spite of this fact the mice had an unchanged haemoglobin content as the increased rate of destruction was compensated by an increased rate of formation if we only disregard a short interval before death. That in the cancerous mouse erythrocytes are synthesized at a strongly enhanced rate is shown by Fig. 10. When labelled glycine was injected to mice after tumour formation, about 4 times as much ^{14}C was incorporated into the haemin of the red corpuscles as in that of controls.

TRACING THE ORIGIN OF BODY CONSTITUENTS AND THE REACTION PATHS

A very fascinating application of radioactive tracers is the locating of the origin of body constituents and the study of the paths of reactions taking place in the organism.

One of the earliest applications of ^{32}P was the tracing of the origin of the phosphatide molecules of the milk in order to find out if these move from the plasma into the milk or are synthesized in the milk gland⁽¹⁷⁾. Labelled orthophosphate was administered to the goat and 4.5 hours later the specific activity of the phosphatide P of the milk, plasma, milk gland, liver and kidney was determined. As seen in Table 1 the milk phosphatide phosphorus could not have originated from the plasma phosphatides as the specific activity of the latter is much lower than that of the former. A precursor can clearly not have a lower specific activity than the product formed from the precursor if we are not in that stage of the experiment in which the specific activity of the plasma phosphatide has already decreased with time. This was not the case in the above experiment, taking 4.5 hours only. Thus it follows from the figures of Table 1 that the milk phosphatides were synthesized in the milk gland. The specific activity of the milk phosphatides is lower than that of the milk gland phosphatides. We cannot expect the milk phosphatides to have exactly the same specific activity as the milk gland phosphatides, we can only expect them to be lower or equal. The phosphatides present in the milk are released at different times from the milk gland and the milk released by the gland was partly built up at a time when the ^{32}P activity level of the gland was very low or even absent. The figures of Table 1 demonstrate also that the phosphatide synthesis in the milk gland is going on at a more rapid rate than in the liver or the kidneys, thus at a very rapid rate.

SEYMOUR COHN's tracing of the origin of the phosphorus atoms of the desoxyribonucleic acid, one of the main constituents of the virus investigated, is a beautiful example of this type of application of radioactive indicators⁽¹⁸⁻¹⁹⁾. In bacteria such as the *Escherichia coli* 80% of the total P

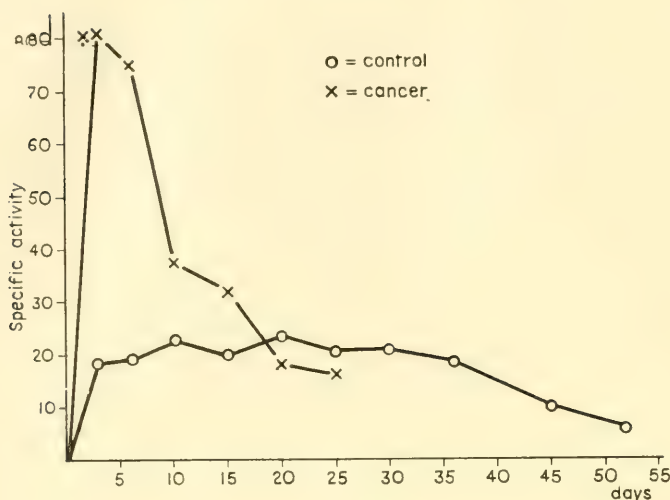


FIG. 10. Loss of ^{14}C by the hemin of red corpuscles of control mice and mice inoculated with ascites cells 10 days prior to injection of glycine- ^{14}C -2. The figure is showing the effect of cancer on the formation of red corpuscles.

content is present in the nucleic acids which are predominantly composed of ribonucleic acid. In the virus T_2 we find DNA to contain almost all phosphorus present. If we infect *Escherichia coli* with T_2 bacteriophage in a synthetic medium during the latent period, at a time when division of bacteria cells had stopped, protein and DNA synthesis will still go on, the newly formed constituents being almost exclusively found in the virus formed as a result of infection. As to the phosphorus atoms of nucleic acid, as seen in Fig. 11, these are to a very large extent found now in the DNA molecules.

If we wish to know the origin of the phosphorus atoms of the virus, whether they are derived from the phosphorus atoms present in the host prior to infection or derived from the medium, we grow bacteria contain-

TABLE 1. — ACTIVITY OF PHOSPHATIDE
PHOSPHORUS OF MILK AND ORGANS OF A GOAT

Fraction	Relative specific activity* per mgm phosphatide P
Milk	1
Plasma	0.02
Milk gland	1.4
Liver	1
Kidneys	1.2

* 4.5 hr after administration of labelled sodium phosphate.

TABLE 2. — DISTRIBUTION OF ^{32}P OF THE BACTERIA BETWEEN VIRUS P AND HOST P IN WITH T_2 BACTERIOPHAGE INFECTED *ESCHERICHIA COLI*

Radioactivity of virus isolated after synthesis in ^{32}P -labelled cells in media free of ^{32}P

Virus isolated	DNA-P	Bacteria counts per 10 γ P per min	Virus counts per 10 γ P per min	Relative radio-activity virus P host P per cent
	Total P in virus			
$\text{T}_2\text{r}+^*$	1.00	222	33	14
$\text{T}_2\text{r}+\dagger$	1.00	293	53.5	18
$\text{T}_4\text{r}+^*$	1.01	37.8	5.9	16
$\text{T}_4\text{r}+\dagger$	1.06	293	53.3	18

ing ^{32}P , infect them with the bacteriophage and compare the radioactivity of 1 microgram of virus P and 1 mgm of host P. As seen in Table 2 the latter makes out 16% only of the former, proving that the bulk of the virus P cannot originate from the host, it must have been taken up from the medium in which it was grown.

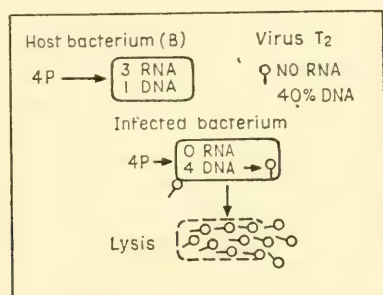


FIG. 11. Shunt in phosphorus (P) utilization and nucleic acid synthesis during virus multiplication.

We can also arrive at this result by following another procedure, i.e. grow virus in unlabelled bacteria and add to the medium labelled phosphate. As seen in Table 3, the activity of 1 microgram of virus P is a very high percentage of the radioactivity of 1 mgm of the phosphate P added as orthophosphate to the medium.

While the application of radioactive phosphorus permits to locate the origin of the virus phosphorus that of ^{14}C labelled glucose leads to very instructive information on the origin of the ribose found in the PNA and the desoxyribose found in DNA molecules. SEYMOUR COHN demonstrated the presence in *E. coli* of two alternative pathways for glucose metabolism as presented in Fig. 12. After the glucose being con-

verted into glucose-6-phosphate the further steps lead either through the phosphogluconate pathway (left) or through the triose phosphate pathway (right). By applying ^{14}C labelled glucose he could demonstrate

TABLE 3. — ^{32}P CONTENT OF THE VIRUS SYNTHETISED IN UN-LABELLED CELLS KEPT IN AN ACTIVE MEDIUM

Radiactivity of virus synthetized in unlabelled cells in media containing ^{32}P

Virus isolated	DNA-P	F. medium counts per 10 γ P per min.	Virus counts per 10 γ P per min.	Relative radio-activity virus P medium P per cent
	Total P in virus			
$\text{T}_2\text{r} + ^*$	1.04	443	315	71
$\text{T}_4\text{r} + \dagger$	1.00	840	633	75
$\text{T}_4\text{r} + ^*$		422	291	69

that RNA is derived from the phosphogluconate path and that after infection of the bacteria with the T_2 bacteriophage the metabolic path of glucose is switched over to the triose phosphate path.

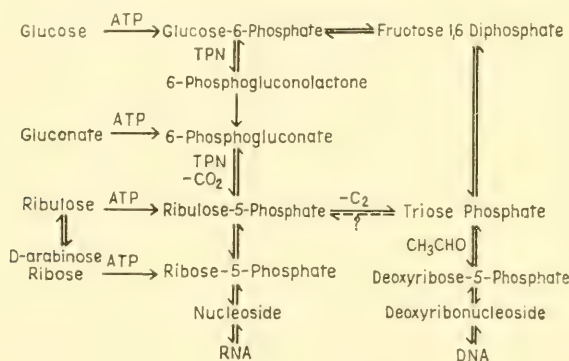


FIG. 12. — Alternative pathways of glucose metabolism prior to and after infection of *E. coli*.

We discussed earlier the effect of insulin on glucose metabolism and also its effect on the transformation of glucose into fatty acids. Here two pathways are found as well, one being the so-called Emden—Meyerhof pathway, the other involving oxydation of phosphogluconate. Experiments with liver slices of diabetic rats treated with insulin brought out that insulin treatment accelerates fatty acid formation from labelled glucose through the Emden—Meyerhof pathway 70 times; through the phosphogluconate pathway however, as much as 375 times.

Lack of time prevents me from discussing another very important and elegant application of radioactive indicators, the tracing of the path of atoms in photosynthesis, which enlarged our knowledge of that important field immensely.

ANALYTICAL APPLICATIONS

The analytical applications of radioactive indicators do not only involve the determination of elements and compounds as, for example, sodium, potassium or water present in the organism but also of particles

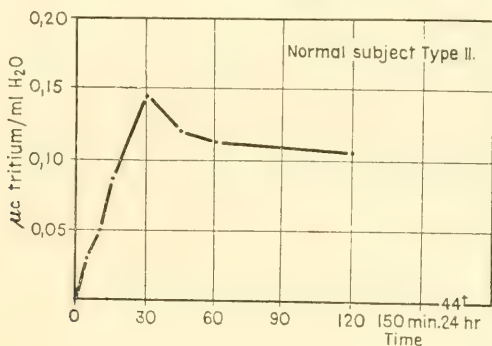


Fig. 13. — Distribution of ingested tritium labelled water in the human.

such as red corpuscles, of the plasma volume, of the extracellular and intracellular water volume and so on. These determinations are all based on the already mentioned dilution principle. Such determinations were for a number of years first carried out by applying heavy water as an indicator.

After the availability of superheavy water, the radioactive tritiated water, it is mostly applied in the determination of the water content of the body. The result of such experiments carried out recently by FALLOT and AEBERHARDT⁽²¹⁾ is seen in Fig. 13. To a fasting subject 100 ml with $^3\text{H}_2\text{O}_2$ labelled water of 10 millicurie of activity is administered. Blood samples are then taken at intervals. They show the largest tritium concentration after 30 min and a constant somewhat lower value after 120 min. That the 30 minutes value is higher than the equilibrium value indicates that the resorption of water molecules from the intestine takes place at a more rapid rate than their equal distribution all over the body water.

Later a decrease in the $^3\text{H}_2\text{O}_2$ content of the body water sets in due to excretion and from this decrease the half-time of the stay of the water molecules in the body is calculated to amount to 9 days by a water intake

of about 3 litres a day. In the described experiments the total dose administered to the subject amounted to 0.9 rep. Such determination can, however, be carried out by administering tritiated water of 1 milli-curie of activity or even less.

Determination of the body water leads also to information as to the fat content of the body. Assuming the lean tissues to contain 73% water the body fat can be calculated from the formula

$$\% \text{ body water} = 0.73 (100 - \% \text{ fat})^{(22)}$$

In the rat the half-time of the water molecules is much shorter than in the human, making 3 days. When following the decrease of the

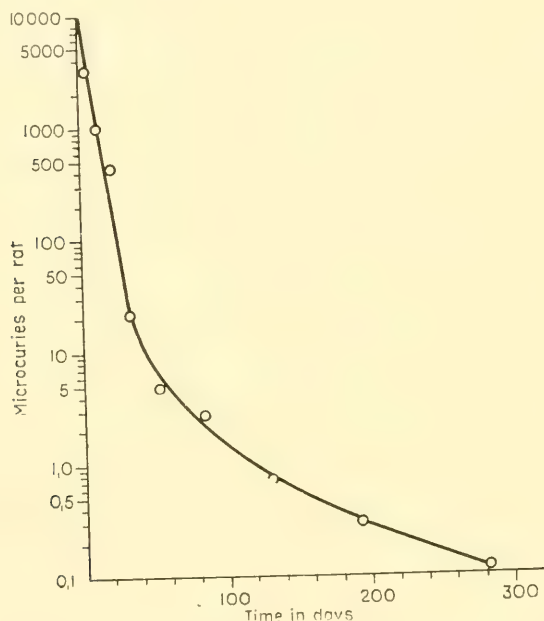


FIG. 14. Decrease of the tritium content of the body water of the rat with time.

$^3\text{H}_2\text{O}_2$ content of the body fluids for many months, as did THOMPSON⁽²³⁾, we have to administer comparatively much larger activities than those mentioned above. He found, as seen in Fig. 14, that while the decrease in the concentration of the tritiated water takes place during the first 40 days in the rat according to an exponential equation with $T_{1/2} = 3$ days, it slows down appreciably after that date. This is due to the following fact: About 2 per cent of the tritium of the administered water finds its way in the course of a day or even less into organic molecules, primarily into proteins, for which we have to correct our water volume figures. Some of these tritium atoms are easily replaced by hydrogen

atoms when the concentration of the former in the water decreases. Some of the tritium atoms incorporated into proteins and other organic body constituents, however, are given off at a very slow rate only and the last part of the curve does not mainly indicate the disappearance of labelled water molecules administered but the disappearance of their hydrogen (tritium) moiety which was hidden in organic molecules and after being released found a new oxygen partner. Thus the second part of the curve does not measure the life-time of water molecules of the body but that of the hydrogen atoms of such molecules.

HAEMATOLOGICAL APPLICATIONS

The dilution method found an extended application in haematological studies. Formerly in such studies the haematocrit figure was considered as an index of the amount of red corpuscles present. When, however, labelled red corpuscles were applied for the determination of the red corpuscle volume, making use of the above mentioned dilution principle, experience brought out that the haematocrit figure cannot be always used to predict the total red corpuscle volume. Figure 15 taken from a paper of Lawrence and BERLIN⁽²⁴⁾ clearly demonstrates that the same haematocrit figure is compatible with very different red corpuscle volumina. We have thus to know, beside the haematocrit figure, the total plasma volume to get information on the total red corpuscle volume or measure directly the latter. We can label the red corpuscles by introducing a radioactive isotope which remains fixed in the corpuscles during the experiment and inject a known volume of these red corpuscles of known activity. After the lapse of 10 minutes, for example, when under physiological conditions, an equal distribution of the injected labelled red corpuscles in the circulation took place, we remove a blood sample and determine the radioactivity of its red corpuscles. From the ratio of the activity of 1 ml injected and 1 ml diluted labelled red corpuscles the volume of the diluting red corpuscles present in the body can be calculated in the same way as the water content of the organism is calculated from the decrease in the heavy water content of the body water after injection of labelled water.

With ^{32}P labelled red corpuscles, were applied by NYLIN and CELANDER⁽²⁵⁾ and with ^{131}I labelled serum albumin by MCINTYRE and assoc.⁽²⁶⁾ in the measuring of the cardiac output for example. This determination is based on the measurement of the varying concentration of the injected material as it passes through the heart. If a small volume of highly concentrated material is injected into the inflow tract, the average dilution of the material coming out from the heart will be a direct indication of the volume passing through the heart and causing this dilution. The

measurement of the change of concentration during a definite interval thus permits the output to be expressed as volume of flow per time unit. By a blood volume of 6.11 litres for example, the dilution curve indicates an output of 5.48 l/min.

The most perfect labelling of red corpuscles is achieved by introducing radioactive iron into the haemin, which however in mammalia only takes place *in vivo* during its formation in the bone marrow. In clinical experiments iron labelled red corpuscles cannot be applied as they necessitate a donor to whom a radioiron sample of an unpermissibly high radioactivity must be administered. In clinical studies in the course of the

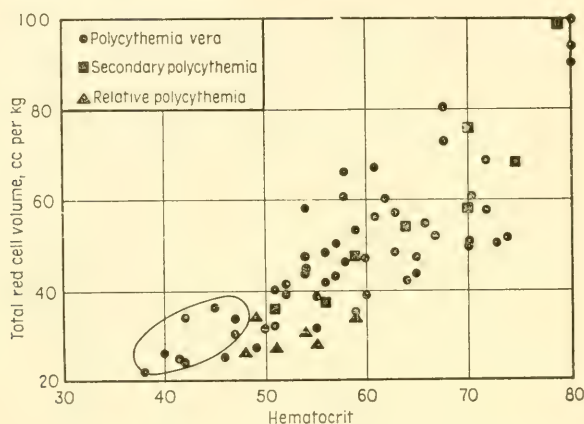


FIG. 15. — Haematocrit plotted against red corpuscle volume.

first decade following the introduction of radioactive labelling of red corpuscles mostly ^{32}P labelled red corpuscles were applied. Occasionally also such labelled with ^{42}K or ThB .

Recently labelled chromate⁽²⁷⁾ is much used in the determination of red corpuscle volume. Chromate is conserved for a long time in the erythrocytes, half of the radioactive label being lost after the lapse of 26 days only. Furthermore by following the radioactivity of chromium labelled red corpuscles for many days we can get information whether their life-span is normal or shortened as in the latter case the half-value of the loss of radiochromium is shortened as well. Important clinical information can thus be obtained by following the rate of release of ^{51}Cr by the *in vitro* labelled red corpuscles reinjected into the circulation. In the labelling with ^{51}Cr , red corpuscles of all ages are involved, and this is also the case when applying ASHBY's differential agglutination technique. It is occasionally of interest to label red corpuscles built up at a certain date, i.e. within an interval of some days only. This can be achieved by administering ^{14}C labelled glycine, 8 out of the 34 carbon

atoms of haemin originating from glycine, an appreciable part of the ^{14}C administered will be incorporated into haemin in the first phase of the experiment. Later, as the specific activity of glycine markedly decreases with time, haemin of minute activity is synthetised only. When the strongly active red corpuscles built up in the first phase of the experiment terminate their life-cycle, they become phagocytised and the circulating red corpuscles show correspondingly a decreased activity as seen in Fig. 16⁽¹⁴⁾ which indicates the life-span of human red corpuscles in this

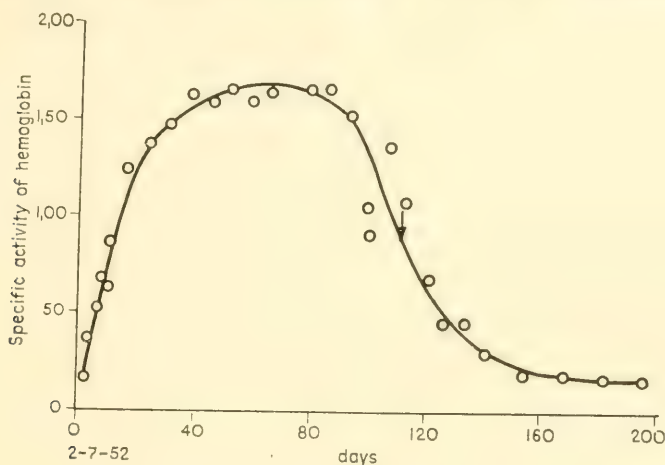


Fig. 16. — Activity of human red corpuscles at various times after administration of glycine- ^{14}C -2.

case to be 113 days. This principle was first applied by RITTENBERG and SHEMIN⁽²⁸⁾ who administered ^{15}N labelled glycine and determined the change in the ^{15}N content of the haemoglobin of the red corpuscles with time, making use of the mass spectrograph.

We mentioned already that in the cancerous organism the life-span of the red corpuscles is often found shortened. The possibility of labelling erythrocytes synthetized within a limited time interval enabled EHRENSTEIN to carry out the experiments described on p. 1008, which permit to distinguish between the action of cancerous plasma on erythrocytes built up in a non-cancerous organism and the imperfection of the latter due to their formation in a cancerous organism.

PERMEABILITY OF PHASE BOUNDARIES

Radioactive tracers found an extensive and fruitful application in the study of permeability of phase boundaries of the capillary wall, the placenta, the blood brain barrier, the frog skin and numerous other cases.

Figure 17 demonstrates the result of the first investigations carried out on the permeability of the capillary wall⁽²⁸⁾. It brought out the very great speed at which ions of the plasma interchange with those of the extravascular space. In the course of 1 minute half of the sodium atoms originally located in the circulation leave the plasma of the rabbit. In the human organism after the lapse of 18 minutes 80 per cent equipartition of the sodium ions, originally present in the plasma is reached⁽³⁰⁾.

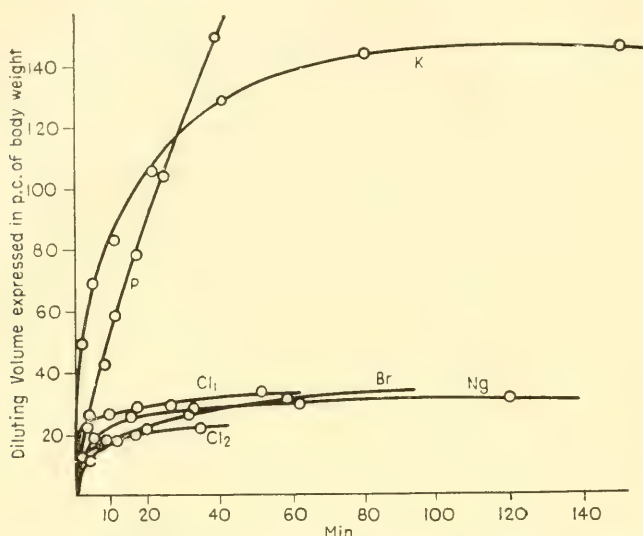


FIG. 17. — Rate of extrusion of intravenously injected labelled ions from the circulation of the rabbit, expressed in dilution volume of body water.

In the early experiments which lead to the results seen in Fig. 17, blood samples were taken at intervals and their radioactivity compared. The great progress made in the construction of measuring devices makes it possible today to follow the disappearance of radioactive sodium from the circulation by placing a scintillation counter in the precordial region and to measure activity changes within extremely short time intervals. Our knowledge on the pattern of disappearance has also been much increased since the first investigations were carried out in this field. We know now that the curve representing the distribution and disappearance of injected radioactive sodium from the plasma is composed of at least 6 components. The first, very rapid component is followed by a second one which was measured by STRAJMAN and his associates⁽³¹⁾ in men and women of different ages. Their very instructive results are seen in Fig. 18. With increasing age, presumably due to an increasing arterial sclerosis, the distribution of the injected sodium ions in the circulation takes place at a decreasing rate.

Such experiments, as those last mentioned can only be carried out by making use of radioactive indicators while many of the clinical analytical determinations discussed do not necessitate the application of isotopic tracers. We can determine the water content of the body by following up the dilution of antipyrin determined by the usual chemical methods, we can label red corpuscles instead of radioactive bodies with carbon-monoxide, the concentration of which is determined chemically, the

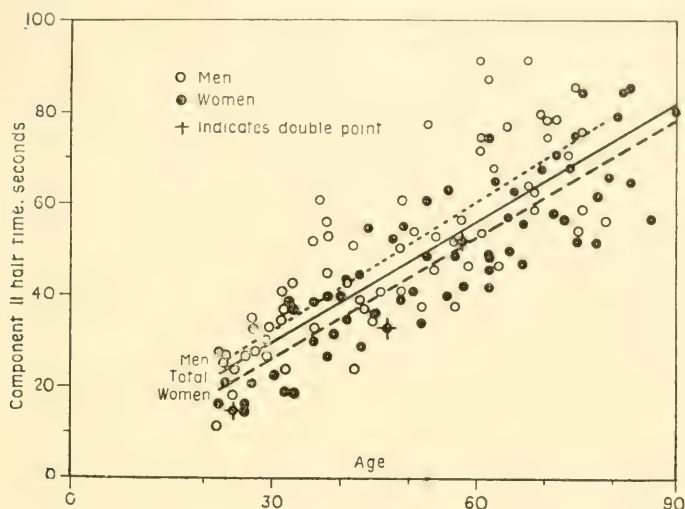


FIG. 18. — Change of the mixing rate of sodium ions in the circulation with age.

plasma volume can be measured by following the dilution of Evans Blue. The application of radioactive tracers in these investigations is not a necessity but a great convenience. Not so when splitting dynamical equilibria, the only way to follow the path of iron in the organism is to label it. The same applies to the tracing of pathways as that of glucose carbon for example, discussed above.

The determination of the life-time of various molecular components of the organism was a great advance, a classical example being the determination of the half-life of saturated and unsaturated fatty acids of the rat liver which, in the early experiments of SCHOENHEIMER and RITTENBERG using deuterium as an indicator and in later investigations in which ^{14}C was applied was found to be 1 and 2 days. Recent investigations brought out, however, that the type of molecules in an organ can have very different life-times. The phosphatide molecules present in the cytoplasm of the liver cells are turned over almost twice as rapidly as those present in their nuclei. It was also found, that in the liver a fraction of fatty acid molecules is renewed at a very much more rapid

rate as stated above, namely with a half-time of about 10 minutes only. Interesting observations were made when rats were kept from their conception until they reached an age of 6 months on water containing $^3\text{HO}_2$ and for the consecutive 10 months on normal water. After that date the various body constituents still retained a significant fraction of their tritium content indicating that a corresponding fraction of the molecules considered were not renewed in the lapse of 10 months. From the saturated fatty acid molecules of the whole body 4% kept their tritium content at that date. It could be followed from these experiments that 60% of the whole body saturated fatty acid molecules have a half-time of 80 days while another fraction has a half-time of 15 days. These data⁽³²⁾ though of great interest, do not claim great accuracy as tritium is a somewhat imperfect indicator of hydrogen, the atoms of the former being three times as heavy as those of the latter.

This great mass difference reflects itself in a corresponding isotope effect. We can best test the accuracy with which the behaviour of deuterium indicates that of hydrogen by comparing the distribution of deuterium and tritium in the compound to be investigated.

If we administer water containing some D_2O and some T_2O and take the T/D ratio to be 1 in the case of an absence of an isotope effect we should find in the water obtained by combustion of any tissue or tissue constituent a T/D ratio of 1 as well. THOMPSON and BALLON⁽³²⁾ investigated the T/D ratio of the combustion water obtained of organs of the mouse 3 days after injecting with deuterium and tritium labelled water and found the following T/D ratios.

TABLE 4.
RETENTION RATIOS (T/D)

Pelt hydrogen	0.98
Muscle hydrogen	0.93
Fat hydrogen	0.99
Brain hydrogen	0.95

the extent to which tritium fails to indicate correctly deuterium we can estimate the error incurred when applying deuterium as an indicator of hydrogen. We arrive at this figure by multiplying by about twice the first mentioned error.

Diluted heavy water containing almost exclusively DHO molecules is a suitable indicator of the HHO molecule (cf. p. 997), not, however, concentrated heavy water containing to a large extent DDO molecules. Concentrated heavy water is a toxic substance.

With increasing atomic mass the isotope effect decreases, it can, however, be still noticeable for carbon. The error incurred by considering ^{14}C as a correct indicator of ^{16}C can be estimated by applying both ^{14}C and ^{13}C as tracers, as tritium and deuterium are applied in the above mentioned case.

That the atoms building up the living organism are replaced in time is common knowledge. The possibility of measuring the rate of their renewal and of following the path they take is a recent advance. A task not easy to perform due to the reasons mentioned above and often the lack of knowledge of the decisive precursor or precursors. New possibilities of experimental attack stimulate our thoughts and lead them into new channels. Problems come into the focus of interest which were not considered previously. Here lies possibly the main importance of the introduction of the method of isotopic indicators.

To demonstrate that the notion of renewal of body constituents is an ancient one, I beg you to permit me to read a statement made in the thirteenth century by one of the greatest sons of this venerable and beautiful country, Thomas of Aquina. What is to happen, asks the saint, to a man who never, throughout his life, ate anything but human flesh, and whose parents did likewise? It would seem unfair to his victims that they should be deprived of their bodies at the last day as a consequence of their greed; yet, if not, what will be left to make up their body? I am happy to say that this difficulty, which might at first sight seem insuperable, is triumphantly met.

The identity of the body, St. Thomas points out, *is not dependent on the persistence of the same material particles*; during life, by the process of eating and digesting, the matter composing the body undergoes perpetual change. The cannibal may, therefore, receive the same body at the resurrection, even if it is not composed of the same matter as was in his body when he died.

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COMMENT TO PAPER 98, 99, 100

THE HISTORY of the application of radioactive tracers covers 5 epochs. From 1913 to 1932 only radioactive isotopes found in nature were applied in tracer studies. From 1933 onwards first diluted, later concentrated deuterium and also concentrated ^{15}N and ^{13}C were available for indicator studies mainly due to the discoveries of Urey. The fundamental discovery of artificial radioactivity by Frédéric Joliot and Irène Curie made it possible to label almost every element. From 1934 to 1937 artificial isotopes produced under the action of neutrons emitted by radium-beryllium sources were applied in the production of radioactive tracers. The year 1937 witnessed the opening of a new epoch due to the availability of strongly active samples produced by the cyclotron. Ernest Lawrence's ingenuity and generosity made these radioactive bodies available.

After the second world war the last epoch of the application of radioactive tracers started. Pile produced radioactive isotopes of almost unlimited activity became available, among others ^{14}C and ^3H . Samples of minute activity of these radioactive bodies were available prior to that date. ^{14}C discovered in 1940 by Ruben and Kamen became the most frequently applied radioactive isotope in life sciences, instead of the formerly most extensively used ^{32}P .

The investigations described in paper 98 were all carried out prior to the advent of the last epoch of tracer studies, in contrast to those discussed in paper 99. In paper 100 some outstanding studies covering all 5 periods are described. It is emphasized that while tracer methods made it possible to measure the rate of renewal of body constituents, the knowledge that such a renewal takes place is far from being a novel one. It goes back to the first part of the thirteenth century and the first to arrive at this knowledge was St. Thomas Aquinas. When pondering about what would happen at the day of resurrection to a man, who had been a cannibal through all his life, and whose ancestors might have been cannibals as well, he arrived at the conclusion that "the identity of the body is not dependent on the persistence of the same material particles", and that "during life, by the process of eating and digesting the body undergoes perpetual changes".

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